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The Journal of Laboratory and Clinical Medicine

VOL. 24 MARCH, 1939 NO. 6

CLINICAL AND EXPERIMENTAL

SPECIFICITY OF PNEUMOCOCCUS TYPES BY QUELLUNG AND AGGLUTINATION REACTIONS*

ARLYLE NOBLE, A.B., AND BERTHA C. CAMERON, B.S., DETROIT, MICH.

CULTURES representative of the 27 types of *Diplococcus pneumoniae*, as established by Georgia Cooper[1, 2] and formerly known as group IV, together with types I, II, and III, were isolated and antisera prepared in rabbits. With these sera and cultures, homologous and cross agglutination and Neufeld Quellung reactions[3] have been studied.

METHODS AND MATERIALS

Cultures.—Sixty-three cultures of *Diplococcus pneumoniae* were used in this study. The samples of sputum from which a large number of these strains were obtained were made available to us through the courtesy of members of the staff at the Receiving Hospital, Detroit, where each sputum had been typed by the Neufeld Quellung test. Within a period of three months cultures representative of the 30 types of pneumococci had been isolated by the mouse method. In this group, material from 65 cases was cultured. From 53 of these, the pneumococcus type corresponding to the sputum diagnosis was recovered; from 12 cases some other type of pneumococcus, Pfeiffer's bacillus or streptococcus was isolated. We were unable to type one apparently typical pneumococcus.

A few additional strains were obtained from sputa and blood cultures from cases of pneumonia at the Henry Ford Hospital. Two or more strains of each type were obtained in all but five (XVII, XXI, XXIII, XXVII, and XXVIII).

All cultures are gram-positive cocci, usually pointed, occurring in pairs and short chains of pairs. They are all soluble in bile; 42 promptly ferment inulin, 21 show slow fermentation, or none at all.

*From the Research Laboratories, Parke, Davis & Co., Detroit.
Received for publication, June 23, 1938.

TABLE I

CROSS QUELLUNG REACTION WITH 30 ORGANISMS USED FOR ANTISERA

CULTURES USED FOR ANTISERA		TYPE SERA																													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	27	28	29	31	32
I	02444	+																													
II	02445		+																												
III	02179			+																											
IV	1170				+																										
V	1171					+																									
VI	1223						+																								
VII	1161							+																							
VIII	1176								+																						
IX	1174									+																					
X	1220										+																				
XI	1216											+																			
XII	1206												+																		
XIII	1202													+																	
XIV	1168														+																
XV	1228															+															
XVI	1186																+														
XVII	1183																	+													
XVIII	1217																		+												
XIX	1222																			+											
XX	1173-1																				+										
XXI	1213																					+									
XXII	1159																						+								
XXIII	1229																							+							
XXIV	1192																								+						
XXV	1207																									+					
XXVII	1233																										+				
XXVIII	1194																											+			
XXIX	1219																												+		
XXXI	1237																				+									+	
XXXII	1214																														+

The blank spaces indicate negative results.

XXXII 1214

The blank spaces indicate negative results.

TABLE II

CROSS QUELLUNG REACTIONS WITH DUPLICATE CULTURES ON CERTAIN TYPES

CULTURES		TYPE SERA																													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	27	28	29	31	32
I	1234	+																													
II	1235-1		+																												
III	1238			+																											
IV	1180				+																										
V	1163					+																									
	1221					+																									
VI	1181						+																								
VII	1154							+																							
	1162							+																							
VIII	1240								+																						
	1241								+																						
IX	1178									+																					
X	1239										+																				
XI	1199											+																			
XII	1195												+																		
XIII	1196													+																	
XIV	1177														+																
XV	1235-2															+															
XVI	1230																+														
XVIII	1203																		+												
	1224																		+												
XIX	1215																			+											
XX	1188																				+										
XXII	1169																						+								
	1175																						+								
XXIV	1191																								+						
XXV	1166																									+					
	1189																									+					
XXIX	1200																												+		
XXXI	1231																				+									+	
	1236																													+	
XXXII	1167																														+
?	1218																														

The blank spaces indicate negative results.

TABLE

CROSS AGGLUTINATION REACTIONS WITH

TYPE SERA	CULTURE USED FOR SERUM	SUSPENSIONS OF DIPLOCOCCUS												
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
		02444	02445	02179	1170	1171	1223	1161	1176	1174	1220	1216	1206	1202
1	02444	*4443												
2	02445		4400						±					
3	02179			4421										
4	1170				4444				±					
5	1171					4410								
6	1223						4420							
7	1161							4300						
8	1176								4444					
9	1174									4444				
10	1220								±		4444			
11	1216								±			4443		
12	1206								±				4444	
13	1202													444
14	1168													
15	1228								±					
16	1186								±					
17	1183								±					
18	1217								±					
19	1222								±					
20	1173-1						±		±		±			
21	1213													
22	1159								±					
23	1229													
24	1192				±									
25	1207													
27	1233													
28	1194								±					
29	1219				±		±		±	±				±
31	1237								±					
32	1214								±	±				
Controls		0	0	0	0	0	0	0	0	0	0	0	0	0

*Four amounts of undiluted serum were used—0.05, 0.025, 0.01, and 0.005 c.c., corresponding to dilutions of 1:20, 1:40, 1:100, and 1:200.

4 = complete agglutination; 3 = marked; 2 = partial; 1 = slight; 0 = no agglutination.

The blank spaces indicate negative results.

The cultures are maintained in rabbit blood (one part defibrinated blood to two parts saline, about 5 c.c. to a tube). Cultures are incubated at 37° C. for eighteen hours and stored at 4° C.

Method of Producing Antisera.—Rabbits, weighing about 5 pounds each, were used throughout. Antigens of each type were prepared by suspending the young growth from ascites agar in saline plus 0.5 per cent formalin. Each rabbit received one dose subcutaneously and then intravenous doses of increasing amounts twice weekly for from three to six weeks. At intervals the rabbits were sample bled from the ear and the serum tested for Quellung reaction and for agglutination. If a clear-cut reaction was not obtained, they were further treated.

The Quellung tests were made on either peritoneal fluid from mice injected with the specific culture or on rabbit blood culture. In either case formalin had been added. The agglutination titers ranged from 1:40 to 1:200 and sometimes higher. The serum from two or three rabbits on each type was pooled and preserved with 0.5 per cent phenol.

Technique of the Quellung Reaction.—Eighteen-hour cultures in rabbit blood preserved with about 0.5 per cent formalin were used for the Quellung

TABLE III

ORGANISMS USED FOR TYPE ANTISERA

PNEUMONIAE—30 TYPES																	
XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	XXV	XXVII	XXVIII	XXIX	XXXI	XXXII
1168	1228	1186	1183	1217	1222	1173-1	1213	1159	1229	1192	1207	1233	1194	1219	1237	1214
		±														
		±														
		±														
		±														
		±														
		±														
		±														
4444		±					±									
444																
440																
	4442	±														
		3211														
		±	4443								±					
		±		4421												
		±			3321											
		±				4444	±					±				
		±					4444									
								4431								
		±							4431							±
		±								4321						
					±						4421					
												4443				
													4431			
±		±					±							4443		
															4444	
														±		4442
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

reactions. Formalin appears to intensify rather than lessen the reaction and makes a culture safe to use. Only a few cultures were prepared at a time and used from two hours to a week after the addition of formalin, though much older cultures have often given satisfactory results.

A drop of Loeffler's methylene blue from a fine capillary pipette is placed near one corner on each of three cover slips, a small loopful (2 mm.) of the culture being tested is placed near another corner on each of the three cover slips, then a large loopful (4 mm.) of type I serum is added to the loopful of culture on the first cover slip, mixed, and the stain drawn in and further mixed. Type II serum is added to the next and type III to the third. The cover slips are then inverted onto a plain glass slide and the procedure repeated with all the type sera. The slides are then examined with an oil immersion lens, using reflected light from a frosted blue glass bulb (100 watt).

When a positive reaction occurs, there is a swelling of the capsule of the pneumococcus and the capsule has a definite outline. In a negative reaction the capsule of the organism appears only as a halo of refracted light. In either case the pneumococcus is stained a deep blue. There is considerable variation as to the size to which a capsule may swell in the different types. For example, XXV has a very narrow capsule in positive reactions, while types III and VIII have very large capsules.

TABLE

CROSS AGGLUTINATION REACTIONS WITH

TYPE SERA	SUSPENSIONS OF DIPLOCOCCUS																
	I	II	III	IV	V		VI	VII		VIII		IX	X	XI	XII	XI
	1234	1235-1	1238	1180	1163	1221	1181	1154	1162	1240	1241	1178	1239	1199	1195	11
1	4443															
2		4311														
3			4432						211*							
4				4421												
5					4444	4443										
6							0000*									
7								4463	4444							
8			3100							4443	4444	1100*				
9												4444				
10													4442			
11									±					4444		
12															4444	
13																34
14																
15																
16																
17																
18																
19																
20																
21																
22																
23																
24																
25									±							
27									±							
28									±							
29							±*									
31																
32																
Controls	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*Tests repeated with same results.

Four amounts of undiluted serum were used—0.05, 0.025, 0.01, and 0.005 c.c., corresponding to dilutions of 1:20, 1:40, 1:100, and 1:200.

The blank spaces indicate negative results.

Technique of the Agglutination Tests.—Agglutination tests were made by a rapid macroscopic method,[4] using measured amounts of undiluted serum and, for suspensions, sediment from broth cultures.

The supernatant growth from a twenty-four-hour rabbit blood culture is used to inoculate tubes of veal broth (proteose peptone, pH 7.8-8.0) which are incubated eighteen hours. Approximately 0.5 per cent formalin is added and the cultures thoroughly mixed. They are then centrifuged, the broth decanted, and the organisms resuspended in broth and formalin. This suspension is thoroughly mixed and allowed to stand for two or three hours in a narrow tube to allow any clumps or debris to settle. The supernatant homogeneous suspension is then removed to a clean tube and diluted with broth and formalin to a density of 5 to 8 billion organisms per c.c.

Test.—One-half cubic centimeter of each type serum is pipetted to the bottom of small tubes (Kahn tubes), and 0.1 c.c. suspension of the culture being tested added to each. For the homologous type the serum amounts are 0.05, 0.025, 0.01, and 0.005 c.c. The racks carrying the tests are inclined to an angle of 45° and slowly shaken for two minutes, 0.5 c.c. saline added, and the results read.

PLICATE CULTURES ON CERTAIN TYPES

PNEUMONIAE—TYPES																	
XIV	XV	XVI	XVIII		XIX	XX	XXII		XXIV	XXV		XXIX	XXXI		XXXII	?
1177	1235-2	1230	1203	1224	1215	1188	1169	1175	1191	1166	1189	1200	1231	1236	1167	1218*
																±
																±
																1
																±
																1
																±
															±	2211
																±
																±
															±	1
																1
													4410*			±
																1
	4442															1
		4300														
																±
			4410	4432											±	1
					4432											1
						4432							4430*			1
																1
							4442	3321								1
									2000						±	2
															±	2
										4443	4410					2
								±								±
																±
												4443	4000*		±	3222
													3444*	1122	±	2
															4442	2
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

EXPERIMENTAL

Cross Quellung Tests.—Each type serum was tested against the 30 type cultures which were used for antiserum production. The results are contained in Table I. In all the tables Arabic numerals represent serum types and Roman numerals the culture types. It will be seen that with the exception of culture No. 1237, type XXXI, which was positive also in type 20 serum, there were no cross reactions.

Among the cultures not used for serum production another strain of type XXXI (No. 1231) was the only culture which showed cross reactions. This was also positive in type 20 serum, while a third culture (No. 1236) was negative (Table II).

Therefore 61 out of 63 cultures gave type specific reactions by the Neufeld Quellung test under the conditions of the experiment.

Cross Agglutination Tests.—Cross agglutination tests with the 30 cultures used for antisera in general showed little crossing (Table III). Types VIII and XVI gave doubtful reactions with the majority of sera at 0.05 c.c. Whenever a doubtful or apparently inconsistent result was obtained, the tests were repeated.

Among the cultures not used for serum production (Table IV) one type III culture (1238) showed partial agglutination in type 8 serum; one type VII (1162) crossed slightly with 3; one type IX (1178) slightly with 8, and one

type XXXI (1231) showed marked agglutination in types 13, 20, and 29. Culture 1181, which was typed as VI by the Neufeld reaction and checked several times, failed to agglutinate in any serum.

One culture (1218) which is morphologically a pneumococcus, ferments inulin, and is soluble in bile, has been consistently negative by Neufeld and shows some degree of agglutination in practically all type sera, but complete agglutination in none.

Agglutination and Quellung Reaction With Types III and VIII.—As there has been considerable mention in the literature concerning the crossing of types III and VIII, a few additional strains were tested, including a culture of type III labeled "R_3" and one of type VIII labeled "Henrique," received from Georgia Cooper, February, 1934, and an old type III, received from Rockefeller Institute in 1922. The results are given in Table V.

TABLE V

AGGLUTINATION AND QUELLUNG REACTIONS WITH TYPES III AND VIII

	CULTURES	AGGLUTINATION								QUELLUNG	
		SERUM 3*				SERUM 8*					
		0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.	SERUM 3	SERUM 8
III	Rockefeller	3	4	4	4	1	–	–	–	+	–
	02179	4	4	2	1	–	–	–	–	+	–
	1238	4	4	3	2	3	1	–	–	+	–
	1299	3	4	4	4	1	1	–	–	+	–
	1300	4	4	4	4	2	1	–	–	+	–
	Cooper R_3	4	4	4	4	3	1	–	–	+	–
VIII	1176	–	–	–	–	4	4	4	4	–	+
	1240	–	–	–	–	4	4	4	3	–	+
	1241	–	–	–	–	4	4	4	3	–	+
	Cooper "Henrique"	–	–	–	–	4	4	4	2	–	+

*Serum 3 was produced with culture 02179 and Serum 8 with culture 1176.

Five out of six strains of type III crossed slightly with type 8 serum by agglutination, and the five type VIII strains showed no crossing with serum 3, while type specific reactions were obtained with all by the Quellung test.

SUMMARY

Cultures of *Diplococcus pneumoniae*, representative of types I, II, and III, and the Cooper types IV through XXXII (except XXVI and XXX which are now grouped as VI and XV, respectively) were isolated and antisera prepared.

These 30 types were found to be remarkably specific by both agglutination and Quellung reactions, the latter being the more specific.

In contrast to the numerous unsuccessful attempts which have been made in the past to separate various species of bacteria into types, the classification of the pneumococci as originated by the late Georgia Cooper has been repeatedly confirmed.

Our appreciation is due to Dr. A. H. Price of the Receiving Hospital and Dr. F. W. Hartman of the Henry Ford Hospital for their kindness in furnishing us with the material from which the cultures were obtained.

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EVALUATION OF H AND O ANTIGENS IN AGGLUTINATION TESTS FOR TYPHOID FEVER*

LUCILE R. HAC, PH.D., CATHERINE S. FLYNN, PH.D., AND C. ALFRED PERRY, SC.D.
BALTIMORE, MD.

THE value of carefully standardized H and O typhoid antigens as proposed by Felix[1] over Widal's (1896) living antigen or the formolized antigen, is generally accepted. However, there is still uncertainty as to (a) what constitutes diagnostic titers with H and O antigens, (b) the effect of antityphoid inoculations on the development and persistence of H and O agglutinins, and (c) the respective value of living and of H and O antigens in routine public health work.

Felix considered a serum titer of 1:200 or more with an O suspension as a positive agglutination reaction for typhoid fever. The titers which other investigators[2-6] have considered to be significant have, however, varied widely. Rarely[7-13] have O titers as high as 1:160 been found in serum of vaccinated individuals unless vaccination against typhoid has been very recent (within six months). Agglutination titers with the O antigen in normal uninoculated persons have usually been low.[4, 11, 14-16] Occasional high titers may have been due to such persons being carriers.

There is, therefore, a definite need for further clarification and evaluation of reactions with these antigens before they can be used to best advantage in routine diagnostic laboratory work.

SELECTION AND PREPARATION OF ANTIGENS

The wide variation in the agglutination titers considered indicative of typhoid fever is due in no small part to the sensitivity of the antigens used. This, in turn, is dependent on the strains of organisms used and the method of preparation of the antigen. In this study an effort was made to select strains of *Eberthella typhosa* of high sensitivity and to prepare them in the most effective way.

O antigens were prepared according to the methods of Gilbert, Coleman, and Laviano,[4] and Mudd.[17] The turbidity of the final suspensions was adjusted to standard No. 3 of the MacFarland nephelometer. No difference was found in the sensitivity of the antigens prepared by either method. Since the Mudd method was simpler, it was used in preparing all other O antigens. Nine different O antigens were prepared.

Antigens prepared by pooling several different strains of organisms seemed to yield somewhat less satisfactory results than did single strains of highly

*From the Bureau of Bacteriology of the Maryland State Department of Health, Baltimore.
Received for publication, June 27, 1938.

sensitive organisms. The pooled antigen showed only the degree of sensitivity associated with the weaker strains. The addition of the more active strains failed to compensate for the weaker ones. The importance of using a highly sensitive O antigen cannot be overstressed.

No difficulty was encountered in securing highly sensitive H antigens from actively motile strains of *Eberthella typhosa*. Several of these were compared, and all yielded similar titers.

H and O agglutination tests were incubated overnight at 52° C. with the level of the serum dilutions above the level of the water in the bath to create convection currents in the tubes. Titers were based on the highest dilution in which a definite (2+ or higher) agglutination reaction was obtained.

SELECTION OF SERA FOR STUDY

Over 3,000 sera were examined in this study. When there was sufficient material, the blood clots were cultured in Kracke-Teasley medium.[18] Preliminary microscopic agglutinations with living typhoid and paratyphoid B antigens were carried out in serum dilutions of 1:40 and 1:80, and those sera which showed definite agglutination (2+ or higher)* after one hour at 37° C. in the 1:40 dilution were then titrated by the tube method for H and O agglutinins in dilutions from 1:20 to 1:5,120. Furthermore, only those sera which showed O titers of at least 1:80 were selected for study since it had been found that *Eberthella typhosa* is rarely isolated from any case with a lower concentration of O agglutinins. All cases reported as typhoid during the course of this study and on which agglutination tests were made, were found to have had an O agglutination titer of at least 1:80. Two hundred and eighty-eight sera were studied in this way. These were divided into three groups (1) 181 sera from reported cases of typhoid, (2) 69 sera from cases not reported as typhoid, although considerable O agglutinins were present, and (3) 38 sera from febrile nontyphoid cases. In addition, sera from a fourth group of 25 persons who had received antityphoid inoculations were studied.

Specimens were received for the most part in Keidel tubes. After removal of the serum for agglutination tests, the clot was cultured. Special blood cultures were also received from some cases and stool specimens from others.

Sera from 99 of 181 reported cases of typhoid had microscopic† as well as H and O titrations carried out in dilutions from 1:20 to 1:5,120. Titers determined by the microscopic agglutination test using a living antigen have been found to bear no constant relationship to either the H or O agglutination titers.

RESULTS OF STUDY

Group I. Cases Reported as Typhoid.—An O agglutination titer of 1:160 or greater was found in over 90 per cent of reported typhoid cases in spite of the fact that many specimens were taken early in the course of the disease.

*4+ = practically all bacilli clumped; 3+ = approximately 75 per cent clumped; 2+ = 50 per cent; + = 25 per cent.

†The microscopic tests were made using standard size loopfuls of accurately diluted serum and antigen suspensions mixed on cover slips and examined microscopically.

H agglutinin titers were found to be much lower than O. Twenty-one per cent (20.9) of the 181 reported typhoid cases had H titers less than 1:160 as compared to 8.8 per cent for O agglutination, whereas only 47.0 per cent had H titers of 1:640 or greater compared to 74.6 per cent with similar O titers (Table I).

In this group of 181 reported typhoid cases, there were 6 (3.3 per cent) which showed an O titer of 1:320 or higher but no H titer at all, and 13 (7.1 per cent) which showed an O titer of 1:320 or higher and an H titer not higher than 1:40. All except three gave microscopic titers comparable to the O titer. *Eberthella typhosa* was isolated from the blood of 61 of the 181 reported cases and from stool or urine specimens of 36 others.

TABLE I

H AND O AGGLUTINATION TITERS IN 181 CASES REPORTED AS TYPHOID

Titer	Blood Positive *E. typhosa* 61 Sera		Stool Positive *E. typhosa* 36 Sera		*E. typhosa* Not Isolated 84 Sera		All Cases 181 Sera	
	O	H	O	H	O	H	O	H
	Per cent		*Per cent*		*Per cent*		*Per cent*	
1:80	11.5	18.0	8.3	22.2	7.1	21.4	8.8	20.4
1:160	6.6	21.3	5.6	13.9	8.3	20.2	7.2	19.3
1:320	6.5	14.7	2.8	16.6	14.3	10.7	9.4	13.3
1:640 or higher	75.4	46.0	83.3	47.3	70.3	47.7	74.6	47.0

Group II. Cases Not Reported as Typhoid.—Sera were examined from 69 cases with O titers of 1:80 or higher which were not reported as typhoid. Of these, 17 (24.6 per cent) gave O titers under 1:160, while the remaining 52, or 75.4 per cent, had titers of 1:160 or greater (Table II). It seems likely that a large number of these may have been unrecognized or unreported cases of typhoid in view of the fact that 71 per cent of the 69 had O titers of 1:320 or greater, while in a group of 38 nontyphoid cases (see Table III) and 25 persons given antityphoid inoculations (Table IV) only 2 (vaccinated) had O titers as high as 1:160. That some of these cases were undoubtedly typhoid is further supported by the isolation of *Eberthella typhosa* from several blood cultures. If these 52 cases were typhoid fever as the evidence indicates, it is possible that nearly a quarter (22.3 per cent) of the typhoid cases (181 + 52) were never reported as such.

TABLE II

H AND O AGGLUTINATION TITERS IN 69 SERA WITH O TITERS OF 1:80 OR GREATER, NOT REPORTED CLINICALLY AS TYPHOID

	O	H
	Per cent	*Per cent*
Under 1:160	24.6	34.8
1:160	4.4	17.4
1:320	14.5	18.8
1:640 or higher	56.5	29.0

Group III. Febrile Nontyphoid Cases.—Sera were examined from 38 nontyphoid cases. On the basis of agglutination reactions, 10 were tularemia, 5 undulant fever, 4 spotted fever, and 3 infectious mononucleosis. In 16 in-

stances in which no diagnosis was made by the physician, the laboratory tests were negative. The O agglutination titer for typhoid was 1:80 or less in all these cases previously (Table III).

TABLE III

CLASSIFICATION AND O TITERS ON 38 FEBRILE NONTYPHOID CASES

DIAGNOSIS	CASES	O AGGLUTINATION
Tularemia	10	Partial 1:40
Undulant fever	5	Partial 1:80
Spotted fever	4	Partial 1:40
Infectious mononucleosis	3	Partial 1:80
Undiagnosed	16	Partial 1:80

Group IV. Vaccinated Individuals.—The sera of 25 individuals who had been given antityphoid inoculations were tested for their O agglutinin titers.* Only 2 of the 25 sera had titers of 1:160. Nine had been inoculated within a year and the rest from one to three years (Table IV).

TABLE IV

H AND O TITERS ON 25 PERSONS GIVEN ANTITYPHOID INOCULATIONS

HISTORY	PERSONS	O TITER
Inoculations within year	9	Highest 1:80
Inoculations one to two years previous	10	Highest 1:80
Inoculations two to three years previous	6	(2) 1:160; (4) 1:80

DISCUSSION

The titer which is accepted as an indication of positive serologic reaction for typhoid fever should be (a) sufficiently high that it will seldom be found in nontyphoid cases, but (b) low enough that it will be of value in early diagnosis. If too high a titer is used, a large number of reactions will have to be reported as suspicious or indeterminate and other specimens obtained. While the examination of multiple specimens is highly desirable, it is not always possible for a physician to obtain these, and he may construe a suspicious report on a typical case as contraindicating the diagnosis of typhoid fever.

When highly agglutinable O antigens have been used, a titer of 1:160 has been found sufficiently high to constitute a positive serologic test for typhoid fever. This titer is found early enough (in the course of the disease) to be practical, and yet is rarely found, after six months, in persons who have been given antityphoid inoculations or who are suffering from infections other than typhoid. In our experience *Eberthella typhosa* has seldom been isolated from any case of typhoid when the O titer has been less than 1:80.† In the vast majority of cases of typhoid, an O titer of 1:160 or greater has been found with the first specimen submitted to the laboratory, and in practically all cases where *Eberthella typhosa* has been isolated from the blood stream, the O titer has been so high (1:640 or greater in 75 per cent of cases) that no reasonable question could be raised as to the diagnosis. In cases where the O titer was

*The authors are indebted to Dr. H. B. Miller, of the 1392nd Company C.C.C. Camp D-2-Md., Goldsboro, Maryland, for blood from vaccinated persons.

†One or two isolations have been made subsequent to this study.

comparatively low on the first specimen submitted, a high titer was invariably obtained in subsequent specimens. (O agglutinins have been found considerably earlier in the course of the disease than the H agglutinins.) In a series of 38 febrile, nontyphoid cases, all of the sera had agglutination titers below 1:160. Furthermore, sera from only 2 of 25 vaccinated individuals had titers as high as 1:160. Our results, therefore, are in agreement with those of Felix,[7] Smith,[2] Gardner,[6] and Lewin,[3] who suggested that a 1:200 titer was diagnostic, or, to be more exact, since our dilutions did not include 1:200, we feel that a titer of 1:160 may be considered diagnostic. The 1:500 titer of Dulaney and others[12] has been found to be too high with the antigens used in this study. Since a rise in titer seems to be the best indication of infection, we would agree with Bole[19] and others that it is best to carry out the complete titration each time rather than to use only one or two dilutions.

Sera from a number of cases of undiagnosed illness were found to have an O agglutination titer of 1:160 or higher. The agglutination reactions were so high in most cases as to leave little question of typhoid infection, and in two cases, *Eberthella typhosa* was isolated from blood cultures. Although these had not been so reported, many of them probably were typhoid fever. It is, therefore, possible that nearly a quarter of the typhoid cases considered in this study were never reported as such. These findings warrant a health department in promptly investigating all cases which show positive serologic tests for typhoid fever and in collecting blood and stool specimens from these cases regardless of whether or not they are clinically positive. This observation is in accord with that of Sellers[20] who estimated typhoid fever cases in Georgia for 1935 on the basis of deaths as 2,500 against only 1,013 reported.

There has been a tendency in recent years to disparage the microscopic Widal test with a living antigen. The use of dried blood is, of course, a crude and inaccurate method, though it has been of very great value. In our study of over 3,000 cases of possible typhoid fever, the microscopic agglutination test carried out with a sensitive, smooth, living antigen has been found to be almost as valuable from a diagnostic standpoint as the macroscopic test using the newer H and O antigens, provided the amount of serum available is sufficient for accurate dilutions and the technical worker has had experience in the reading of the microscopic test. Occasionally, the O titer is considerably higher and hence of greater diagnostic value than the titer with the living antigen, although at times the reverse may be the case. H and O antigens can be carefully standardized, readings are less susceptible to individual error, difficulties in antigenic and cultural variations can be avoided, and the O antigen is agglutinated earlier in the course of the disease. In addition, the O antigen has been found of value in detecting carriers.

The routine use of an H antigen for typhoid has not been found of practical value. Its use is indicated in those cases in which a vaccinated individual is suspected of having typhoid. Cruickshank[21] has pointed out that H agglutinins have frequently been found to be absent in typhoid cases. While we have not observed this phenomenon to any extent, we have not found the routine use of H agglutination of value. The recent work of Damon[22] confirms

the work of others that H agglutination is not always specific. Also, the H agglutination may, at times, be partly or totally an anamnestic reaction.

Repeated blood specimens have been found to be of great value in cases with comparatively low initial titers or in cases in which there is some question as to diagnosis. We have failed, however, to obtain positive blood cultures in many typhoid cases which have almost invariably given high O agglutination titers with our sensitive antigens.

Nearly all of the 181 sera received from reported cases of typhoid in Maryland, including 61 sera from which positive blood clot cultures had been obtained, showed at least partial O agglutination in a dilution of 1:80. H agglutinins were present in a titer below 1:80 in 7.1 per cent of the cases and entirely absent in only 3.3 per cent of the cases.

SUMMARY

On the basis of this study, an O agglutination titer of 1:160 with an antigen of satisfactory sensitivity may be considered as a positive serologic test for typhoid, while a titer of 1:80 is suggestive and should be checked by further specimens.

Of approximately 3,000 sera examined during 1935 and 1936, 313 were selected on the basis of agglutination reactions with a sensitive living antigen and further studied for their H and O titers.

Sera were examined from 181 reported typhoid cases. In 61 of these, *Eberthella typhosa* was cultured from the blood; in 36 others, from stool specimens. All sera had at least a 2+ O agglutination in a dilution of 1:80. Ninety-one and two-tenths per cent had an O titer of 1:160 or greater, 84 per cent a titer of 1:320 or greater, and 74 per cent a titer of 1:640 or greater.

Of the 61 sera confirmed by positive blood clot cultures, 75.4 per cent showed an O titer of 1:640 or greater, 81.9 per cent an O titer of 1:320 or greater, and 88.6 per cent an O titer of 1:160 or greater.

The titers on sera from 38 febrile, nontyphoid patients and from 25 individuals who had received antityphoid inoculations, were less than 1:160, except in two of the vaccinated individuals.

In routine work, H titrations have not been found of value, except with sera from individuals known to have been vaccinated.

Even though reasonably reliable results can be obtained by experienced workers with the microscopic Widal test using satisfactory living antigens, there are so many practical advantages in the use of an O antigen suspension that it should be routinely employed.

The authors wish to acknowledge the valuable criticisms of this paper made by Elizabeth Petran and Ruth Myers of this department and by Thomas C. Grubb, formerly of this department.

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OBSERVATIONS ON THE USE OF QUINIDINE SULFATE IN CHILDREN*

CHARLES R. MESSELOFF, M.D., NEW YORK, N. Y.

THE action of quinidine sulfate on the human heart has been the subject of numerous investigations. These have established (1) that quinidine produces definite electrocardiographic changes;[1-7] (2) that it is rapidly eliminated from the cardiac structures;[5-9] and (3) that little cumulation of the drug takes place in the heart.[5, 6] Due, no doubt, to the fact that the more serious types of cardiac arrhythmias are infrequent during childhood, studies on the use of quinidine in man have been limited to adults. However, it was deemed desirable to know whether the observations relating to the action of the alkaloid on the human adult heart are applicable to that of the child. Accordingly, the effects of this drug, at various dosages, were studied on 12 children: 10 with regular sinus rhythm, 1 with frequent and persistent premature auricular contractions, and 1 with bundle branch block associated with congenital heart disease.

These observations revealed four significant points with reference to the use of quinidine during childhood: (1) Impairment of A-V conduction and of intraventricular conduction as well as decrease in the amplitude of the positive T-wave, phenomena noted in adult studies,[4, 6, 7] are also seen in children. (2) Quinidine may be safely administered to a child in doses proportionally very large for the age and the weight of the subject. (3) The principles of dosage and of cumulation governing the clinical use of the drug in children appear to be identical with those reported in adults. (4) A further point of interest is the fact that intraventricular conduction in the child with the bundle branch block was not unduly impaired by quinidine. The details of the observations made on the 12 children, together with a discussion of several features of special interest, form the subject of the present report.

OBSERVATIONS

1. *Observations on Children With Regular Sinus Rhythm.*—Quinidine was administered to 10 children with rheumatic heart disease who were attending the Cardiac Clinic. Their ages ranged from 8 to 14 years. All had regular sinus rhythm and none presented signs of congestive heart failure. The initial dose in every case was 9 grains daily, and this was gradually increased until they were taking 45 grains daily in divided doses. Despite the large doses employed, extracardiac evidence of quinidine toxicity, gastrointestinal, visual, or auditory, did not appear in a single instance.

*From the Cardiac Clinic of the Hospital for Joint Diseases.
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The earliest and most constant electrocardiographic change was a decrease in the amplitude of the positive T-wave in all leads. This occurred in every child in this group and appeared to be a very sensitive reaction to quinidine therapy. In some cases it was the sole evidence of quinidine effect on the heart, and appeared with doses as small as 9 grains daily in a few children.

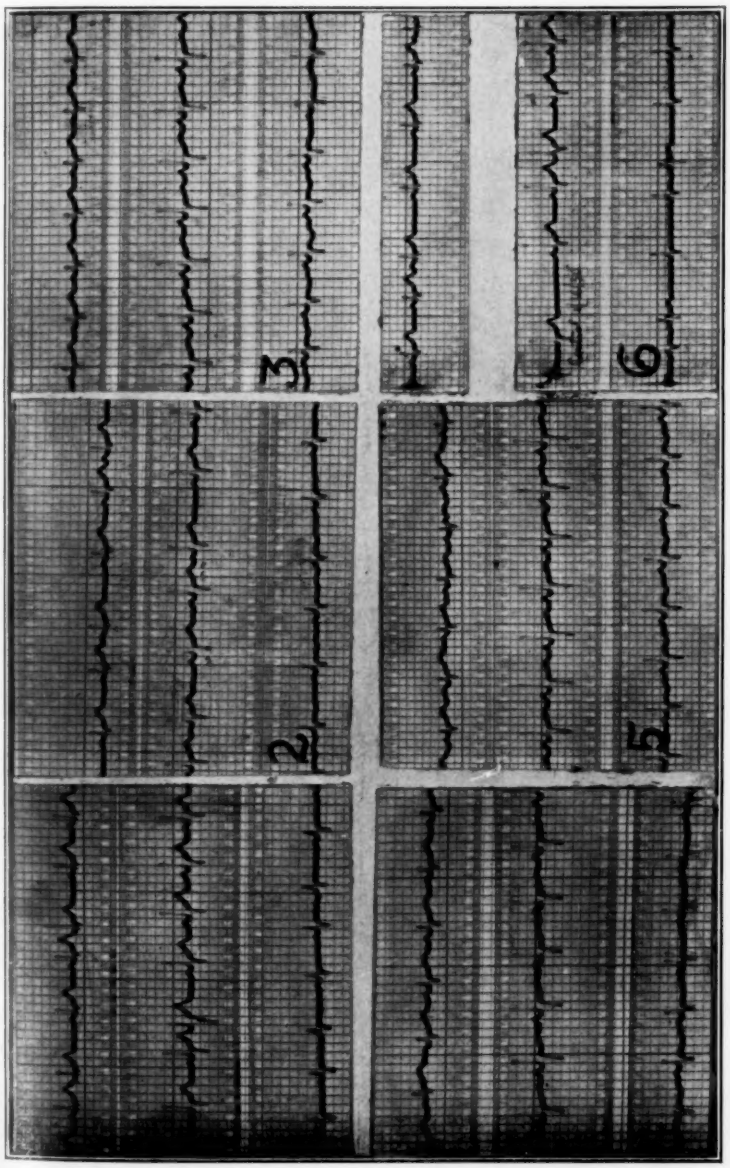

Fig. 1.—Electrocardiograms of patient C. H., showing effect of quinidine. *1*, Before quinidine; *2*, after 15 grains daily for one week; decreased amplitude of T-wave; *3*, after 30 grains daily for one week; abolishment of premature auricular contractions, prolonged QRS time, further depression of T-wave; *4*, after 30 grains daily for two weeks; maximum effects on QRS time and T-wave; *5*, after 30 grains daily for five weeks; persistence of effects noted after two weeks of medication; *6*, after discontinuance of quinidine for two weeks; return of QRS time and T-wave to normal values and reappearance of the premature auricular contractions.

Prolongation of the QRS time, ranging from 25 to 50 per cent of the control values, was present in 4 of the 10 cases. This increase of the QRS time appeared only when the level of dosage was stepped up to 30 grains. A-V conduction was found impaired in two instances, and then only during the period of dosage of 21 to 30 grains daily. In one case the control P-R interval

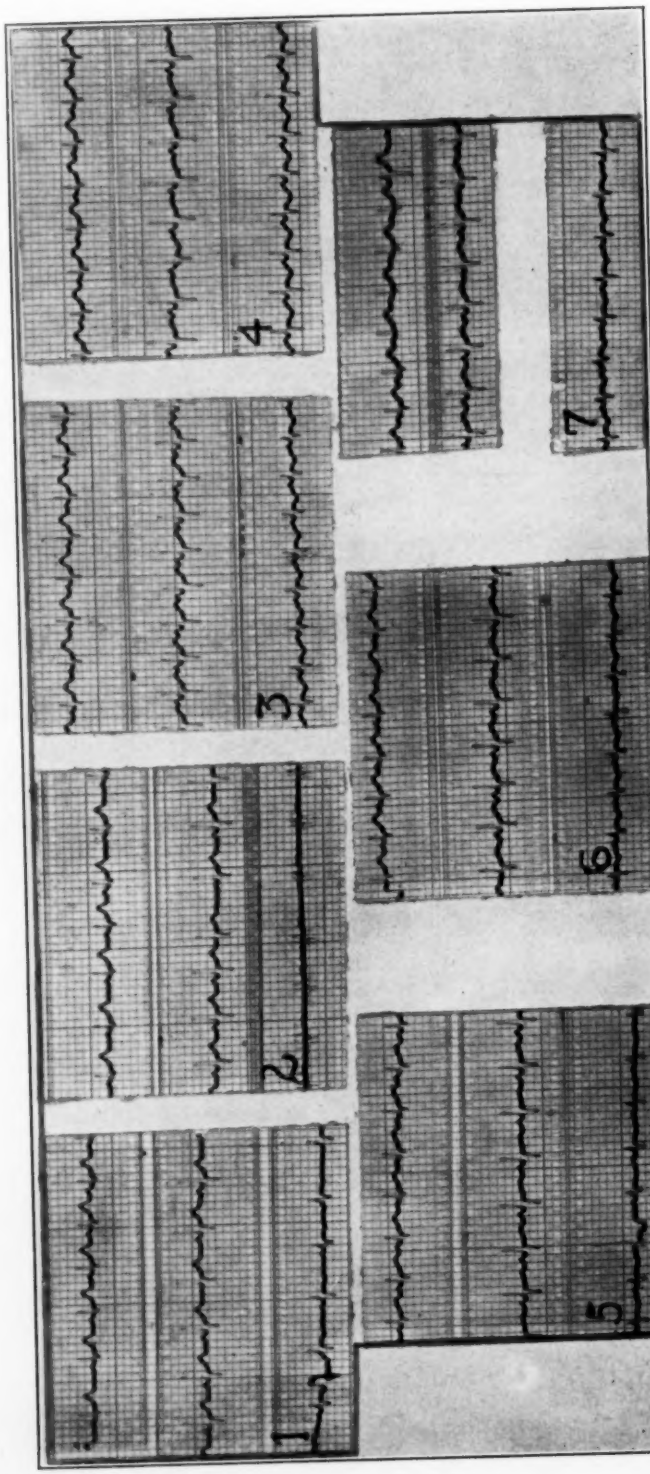

Fig. 2.—Electrocardiograms of patient C. H., showing the effect of a single 15 grain dose of quinidine. *1*, Before quinidine; *2*, thirty minutes after quinidine; *3*, one hour after quinidine; *4*, two hours after quinidine; maximum depression of T-wave; *5*, three hours after quinidine; T-wave still almost isoelectric, ventricular premature contraction; *6*, four hours after quinidine; amplitude of T-wave returning to normal; *7*, five hours after quinidine, further increase in the amplitude of the T-wave, ventricular premature contraction.

of 0.16 increased to 0.18 second, and in the other case the control P-R interval of 0.16 increased to 0.22 second.

2. *Observations on a Child With Premature Auricular Contractions.*—C. H., a boy 10 years old, was referred to the Cardiac Clinic for a study of a cardiac arrhythmia discovered in the course of a routine physical examination. The final diagnosis established in the clinic was regular sinus rhythm with premature auricular contractions, Class E (possible heart disease).*

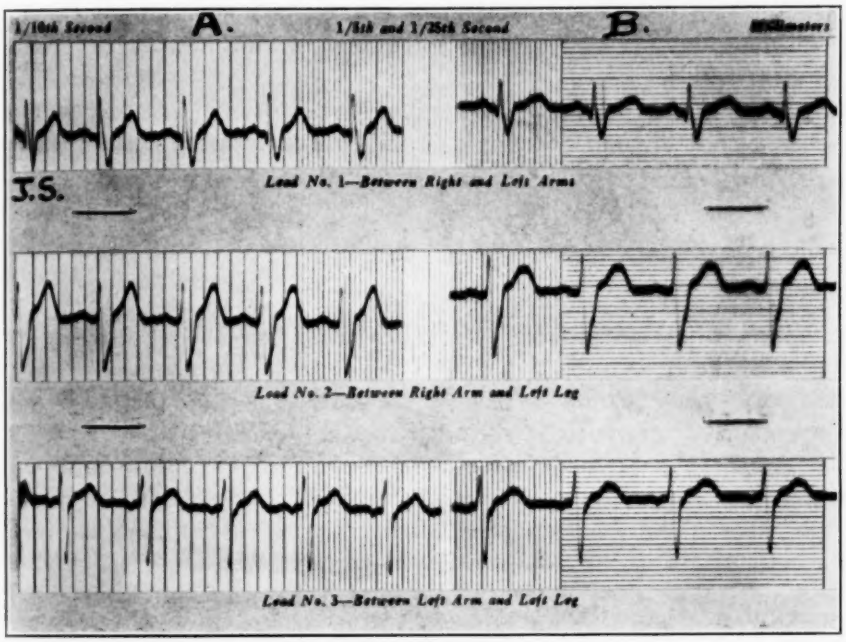
1/10th Second
A.
1/5th and 1/25th Second
B.
J.S.
Lead No. 1—Between Right and Left Arms
Lead No. 2—Between Right Arm and Left Leg
Lead No. 3—Between Left Arm and Left Leg

Fig. 3.—Electrocardiograms of patient J. S. *A*, Before quinidine, shows P-R interval of 0.16 second and QRS time of 0.12 second; *B*, after 30 grains quinidine daily for four days, shows P-R interval of 0.18 second and QRS time of 0.16 second.

The effect of the drug on the premature contractions is shown in Figs. 1 and 2. These show that when quinidine was administered in divided daily doses, it was necessary to increase the level of daily dosage to 30 grains in order to abolish the premature contractions. On the other hand, one single dose of 15 grains sufficed to abolish the ectopic beats within one hour and to prevent their reappearance for at least five hours (Fig. 2). The clinical results of quinidine administration in this child differed in no appreciable way from those obtained in adults with a similar arrhythmia.[10-12]

Electrocardiographically, there was evidence of depression of both A-V and of intraventricular conduction during the period of dosage of 30 grains daily, the P-R interval having increased 29 per cent and the QRS time 25 per cent above the original values. A lowering of the amplitude of the positive T-wave appeared after one week of 15 grains daily, while the maximum decrease of about 50 per cent below the control reading appeared only at the level of daily dosage of 30 grains.

*Following the nomenclature adopted by the New York Heart Association.

The maximum effects of the single dose of 15 grains consisted of a 27 per cent decrease in the amplitude of the positive T-wave and a 10 per cent increase in the QRS time (Fig. 2). These changes appeared within two hours, which is about the same time required in adults for a similar dose to produce its greatest effect.[2, 6] The electrocardiographic changes seen in this child, both during the period of divided daily doses and after the single large dose, were essentially the same as those observed in adults.[6] Attention should, however, be called to the fact that others[13] have failed to find impairment of either A-V or of intraventricular conduction after the administration of as much as 60 grains of quinidine daily. An explanation of this difference cannot be offered at this time.

3. *Observations on a Child With Bundle Branch Block.*—J. S., a boy 6 years old, was referred to the Cardiac Clinic with a diagnosis of congenital heart disease. The diagnosis after complete examination was heart disease of congenital origin, interventricular septal defect, enlarged heart, normal sinus rhythm, bundle branch block, and no diminution of the functional capacity (Class 1).*

In order to observe the effect of quinidine on a heart with a bundle branch conduction defect, the drug was given in gradually increasing doses, beginning with 6 grains daily. It was found that doses up to 9 grains daily failed to produce any change in the electrocardiogram, while doses of 15 and 20 grains daily caused a prolongation of the QRS time by about 17 per cent. A dose of 30 grains daily for four days further increased the QRS time up to 33 per cent above its original value (Fig. 3). No significant changes were seen in the P-R interval, the T-wave, or the sinus rate. These findings are in complete accord with those of Gold and his co-workers[6] who observed no unusual susceptibility of an adult with bundle branch block, given large doses of quinidine, to the depression of intraventricular conduction.

COMMENT

The average daily dose of quinidine in adults is from 15 to 30 grains, although 60 grains and more have been administered daily for long periods of time without untoward effect.[6, 7, 14] The average adult daily dose was exceeded in every one of the 10 children with regular sinus rhythm, 45 grains of the drug having been taken daily for two weeks. None of them showed symptoms of cinchonism or developed serious conduction disturbances or arrhythmias. These observations suggest that the full average adult dose may be safely given to children rather than the fraction of this dose based either on the age or on the weight of the subject. Nevertheless, since great individual variation in sensitivity to quinidine is known to exist, it is advisable to check the degree or the presence of conduction impairment by frequent tracings when large doses are employed, as was done in the present study.

No appreciable cumulation of the drug in the cardiac structures was in evidence at any time. Thus, the daily administration of 30 grains daily for five weeks to the child with extrasystoles produced no greater changes in his electrocardiogram at the end of this period than after one week of medication.

*Following the nomenclature adopted by the New York Heart Association.

However, it should be emphasized that inasmuch as observations were made only at weekly intervals, it is not surprising that cumulation was not noted in any of the cases studied, since cumulation on a fixed daily dose occurs only during the first few days of medication.[6]

It should be further noted that a study of Figs. 1 and 2 shows very clearly that it is the size of the individual dose and not the total period of dosage that determines the magnitude of the effects of quinidine. Thus, one 15 grain dose was more effective in abolishing the premature auricular contractions and was productive of greater electrocardiographic changes than a similar amount given in 5 grain doses three times daily for one week. Moreover, the clinical and electrocardiographic changes produced by the single large dose were practically identical with those noted after the prolonged administration of 30 grains daily but divided into three 10 grain doses.

The findings in the children studied, both as they relate to dosage and to cumulation of quinidine, are essentially the same as those reported by Gold and his co-workers[6] in their study on the use of the alkaloid in ambulatory adult cardiac patients.

SUMMARY AND CONCLUSIONS

1. Observations were made on 12 children relative to the action of quinidine sulfate upon their hearts. The results, clinical and electrocardiographic, were compared with published findings in adults.

2. The electrocardiographic changes produced by the drug in children were found to be identical with those noted in adults. The clinical results in a child with frequent premature auricular contractions differed in no appreciable manner from those seen in an adult with a similar arrhythmia. Published observations that quinidine produced no unusual depression of intraventricular conduction in an adult with bundle branch block were confirmed in a child with a similar conduction impairment.

3. Children tolerate large doses of quinidine, as judged by the age and weight standards. The usual adult daily dose of 15 to 30 grains may be safely given to children. It is advised, however, to control the large doses by frequent electrocardiograms.

4. The principles underlying dosage and cumulation of quinidine established for adults apply equally to children.

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1750 GRAND CONCOURSE

THE EFFECT OF BANANAS ON LAXATION*

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PRELIMINARY to a clinical investigation concerning the laxative effect of various fresh fruits, a short exploratory study was carried out on rats. Normal male albino rats, 90 to 100 days of age and representing eight different litters, were used. The banana was chosen for study as a typical fresh fruit which is readily available, widely used, and of constant composition. Furthermore, several conflicting opinions which have been expressed regarding the laxative value of bananas, should be reconciled if possible. Bergeim and coworkers,[1] Eddy,[2] Russell,[3] and von Meysenbug,[4] have proved to their satisfaction that bananas have a definite laxative value. Other investigators, Thompson,[5] Northmann,[6] Pease and Rose,[7] are equally convinced that this fruit has no influence on bowel movement. However, the American Medical Association, Committee on Foods,[8] has allowed the advertising claim that "Ripe bananas . . . are an aid against constipation," thereby recognizing that bananas have at least a regulating effect upon laxation.

The present experiment was divided into three periods of fourteen days each. During the first period the animals were fed the breeding colony diet consisting of ⅔ whole wheat, ⅓ whole milk powder, and 1 per cent sodium chloride. This same ration plus 20 gm. of banana pulp from fruit not completely ripe (yellow peel with green tip) was fed during the second period. The third period was identical with the second, except that the banana pulp was from fully ripe bananas (yellow peel with brown flecks). One week was allowed between periods II and III, during which the rats received only the basal diet so that no effect of the second period would be carried over to the third.

Of the six criteria for determining degree of laxation proposed by Cowgill,[9] only three were considered practical for use in this study, viz., (1) amount of

*From the Medical College of Charleston and the Research Laboratory, United Fruit Co., New York.

intestinal content evacuated per day, (2) water content of the feces, and (3) laxation rate or number of defecations per day. Consequently, each day at the same hour feces from each individual animal were collected, counted, and weighed. At the end of each week, during the experimental periods, the total collected feces from each rat were dried, total solids were determined, and water content was calculated. The results are shown in Table I.

TABLE I

SHOWING THE LAXATIVE EFFECT OF BANANAS WHEN FED AT DIFFERENT STAGES OF RIPENESS

	FEEDING PERIOD*				
	I	II		III	
	AVERAGE OF RESULTS	AVERAGE OF RESULTS	PER CENT INCREASE OVER PERIOD I	AVERAGE OF RESULTS	PER CENT INCREASE OVER PERIOD I
Laxation rate (number fecal units per day)	15.9	20.9	31	23.9	50
Weight of feces (mg. per day)	1230.0	1760.0	43	1940.0	58
Dry weight of feces (gm. per week)	11.5	12.9	12	14.2	23
Water content (per cent)	33.2	47.6	43	47.7	44
Increase in body weight (gm.)	18.0	24.0	33	38.0	111

*Feeding period I—Animals received the complete colony diet for fourteen days.

Feeding period II—Animals received the colony diet plus twenty gm. of banana (yellow skin with green tip) daily for fourteen days.

Feeding period III—Animals received colony diet plus 20 gm. of banana (yellow peel flecked with brown) daily for fourteen days.

From a consideration of these data it appears that bananas, either partially or fully ripe, do exert a laxative effect. This effect in rats is probably not due to the crude fiber of the bananas, since the 20 gm. of banana pulp contained less crude fiber than the equicaloric amount of basal diet for which it was substituted. Neither does it seem probable that the vitamin B_1 or mineral content of the banana supplements was the factor which produced increased laxation, since the basal diet was liberally supplied with both of these essentials.

A consideration of the relative effects of partially ripe and completely ripe bananas upon the laxation criteria indicated that the laxation principle is present in larger quantities in completely ripe than in partially ripe bananas. The differences in chemical composition of bananas at these two different stages of ripeness are small. Slightly less starch and free tannin, and slightly more sugar and pectin occur in the completely ripe fruit. The small relative amounts of starch and sugar which the banana supplement introduced into the diet eliminate these as causative factors. The free tannin content decreases and the pectin content increases as the banana ripens because the tannins are slowly bound in an insoluble, supposedly inert, "vegetable tannate," while the pectins are slowly freed from an insoluble, inert protopectin. Consequently, the presence of more bound tannin and more free pectin in the fully ripe banana than in the partially ripe fruit should be considered in explaining the superior laxative potency of the fully ripe fruit.

The accelerated gain in body weight during the second and third periods is an anomaly difficult to explain, since the food energy intake increased only slightly during these periods.

Occasional reports[10] of subjective impressions that the laxation benefits from bananas decreased after the first few days of exceptional success in a few human

subjects, were investigated and tested in these experiments. The laxation rates, water content of the feces, and daily amount defecated were averaged for the first and second half of each period and compared in Table II.

TABLE II

A COMPARISON BETWEEN THE RESULTS OF THE FIRST AND SECOND HALF OF EACH EXPERIMENTAL PERIOD

	PERIOD I (CONTROL)			PERIOD II (PARTIALLY RIPE BANANAS)			PERIOD III (FULLY RIPE BANANAS)		
	FIRST HALF	SECOND HALF	PER CENT CHANGE	FIRST HALF	SECOND HALF	PER CENT CHANGE	FIRST HALF	SECOND HALF	PER CENT CHANGE
Laxation rate (number of fecal units per day)	15.3	16.6	+ 8	21.4	20.5	-4	26.2	21.6	-18
Weight of feces (mg.)	1140.0	1320.0	+16	1790.0	1730.0	-3	1960.0	1920.0	- 2
Water content of feces (per cent)	33.0	33.4	+ 1	46.9	48.3	+3	46.7	48.7	+ 4

The subjective impression, that the ability of bananas to increase daily defecations decreased during the course of the experimental periods, was not substantiated in these experiments on rats. In only one instance, the laxation rate during period III, was the performance during the second half of the period markedly decreased over that of the first half.

CONCLUSION

The results of this experiment indicate three facts that may be of value in planning future clinical studies concerning the effect of bananas upon laxation. These are (1) that fully ripe bananas (yellow peels flecked with brown), are more laxative than partially ripe bananas (yellow peels with green tips); (2) that free pectin and bound tannin may prove to be the principal laxation factors of the banana and consequently the intake of these compounds should be determined in both the control and experimental periods; and (3) that there is apparently a sustained increase in laxative effect due to banana feeding in the rat which may also occur in human subjects. Nevertheless, a clinical study should be extended enough so that a relatively great preliminary stimulating effect upon laxation, if present, would not be given undue weight in the final averages.

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RAGWEED HAY FEVER: THE EFFECT OF DOSAGE IN THE TREATMENT OF 314 CASES*

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SPECIFIC pollen therapy is generally accepted as the modern method of treatment of hay fever. The procedure is fairly well standardized and the results of treatment, as tabulated in recent years, are generally accepted to indicate that which might be expected from an average case receiving average treatment with any of the various extracts now obtainable. From the various reports of results of specific pollen therapy, the present method of treatment is likely to yield from 80 to 85 per cent satisfactory results, with 5 to 10 per cent of all cases uninfluenced by therapy.

A study of the literature, however, reveals a variety of factors, which influence the results of such treatment. Among these factors are mentioned (a) the type of treatment: perennial versus preseasonal; (b) dosage: high or low; (c) route of treatment: subcutaneous or intracutaneous; (d) the type of pollen extract used. A special study has therefore been made of a fairly large group of hay fever patients to determine the relative merits of these factors in the treatment.

Rackemann,[1] reviewing the results of the treatment of ragweed hay fever, concluded that larger doses, in the main, produced better end results; that excessive dosage was the cause of failure in a small percentage of the cases; and that the results of several years of treatment were little influenced by other factors, such as pollen counts, etc. Cohen, Reicher, and Breitbart[2] reported better results in the treatment of hay fever with large doses, but this procedure required a greater number of injections for the season. They believed that the main cause of failure was the inability to produce a tolerance to sufficiently large dosage of pollen extract required for protection against the concentration of pollen in the air during the ragweed season. Ramirez[3] concluded, on the basis of a large series of cases, that good results depended upon the administration of large amounts of pollen extract before the onset of the season. Markow and Spain[4] emphasized the importance of high levels of dosage. Eyermann[6] used small dosage in his treatment, but concluded that one of the causes of failure was inadequate dosage. Brown[7] was very emphatic in maintaining that the results of treatment of seasonal hay fever are directly proportional to the size of the maximum dose obtained before the onset of the season. He used a top dose of 60,000 to 100,000 Noon pollen units (1 c.c. of a 6 per cent to 10 per cent

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pollen extract). Similarly, Waldbott,[5] and Waldbott and Ascher[8] found that a maximum amount of relief was obtained with maximum doses, usually of about 15,000 Noon units. On the other hand, Clarke[9] obtained optimal results with a low top dosage (800 Cooke units or 0.015 mg. of total nitrogen) with no ragweed injections during the season. The general consensus of opinion seems to be that the perennial method of treatment gives about 10 per cent better results than the preseasonal method,[10-12] with only an occasional dissenting opinion.[13]

In this study, a review of a large group of hay fever cases, involving five years of treatment, has been made. The factors involved in this study were chosen mainly for their influence on results of treatment and consisted of the route of injection, the method of treatment (preseasonal and perennial), and the various top dose levels.

TABLE I

COMPOSITE CHART

1933 to 1937 Inclusive

		NUMBER OF CASES	PER CENT PERFECT RESULTS	PER CENT GOOD RESULTS	PER CENT POOR RESULTS
ALL CASES		314	5.2	78.9	16.0
Method of treatment	Preseasonal	239	2.9	77.9	19.2
	Perennial	75	12.0	82.7	5.3
Type of extract	Defatted ragweed	108	3.7	78.7	17.6
	Whole ragweed	206	5.8	79.2	15.0
Top dose for season	0.01 mg. N per c.c.	35	0	62.9	37.1
	0.05 mg. N per c.c.	105	2.9	76.1	21.7
	0.1 mg. N per c.c.	113	3.5	86.8	9.7
	Over 0.1 mg. N per c.c.	61	14.8	78.6	6.6
Method of injection	Intracutaneous	210	5.9	79.8	14.3
	Subcutaneous	104	3.8	77.0	19.2
Sex	Males	186	3.2	82.9	13.9
	Females	128	7.8	73.4	18.8
Degree of skin sensitiveness	Class A	5	0	80.0	20.0
	Class B	104	1.9	80.8	17.3
	Class C	205	6.8	78.1	15.1

The patients received daily symptom cards, upon which they recorded the degree of hay fever symptoms for each day of the season. These were checked each week upon their appearance at the clinic, and corrected whenever necessary. All patients were tested by the intracutaneous method and classified according to Vander Veer.* Each patient was tested and retested at the beginning of the treatment, and when necessary, further testing was done to determine the degree of sensitiveness. Two types of extracts were employed: the regular ragweed extract, made according to the method of Coca, and a whole ragweed extract similarly made, except that it was not defatted. Injections were given weekly, and an attempt was made to reach the top dose for each class by the onset of the season. This was impossible in many cases because of a late start and irregularity of attendance. However, this study includes all cases having had sufficient treatment for the purpose of tabulation. Arbitrarily they have

*Described by Vander Veer, Cooke, and Spain in the Am. J. M. Sc. 174: 101, 1927.

been divided into four groups. The first group included those cases that failed to receive doses higher than 0.01 mg. of total nitrogen;* the second group received the top dose of 0.05; the third group received the top dose of 0.1; and the fourth group included all cases that exceeded the top dose of 0.1.

The common denominators in this group of patients were: (1) they were all ragweed hay fever cases, both clinically and on skin tests; (2) they were all treated with ragweed pollen extract, either defatted or nondefatted.

The variable factors that were considered were as follows: (1) sex, (2) method of treatment (preseasonal or perennial), (3) dosage, (4) type of pollen extract used, (5) route of treatment (subcutaneous or intracutaneous), and (6) degree of sensitiveness of patient as revealed by intracutaneous testing.

RESULTS AND COMMENTS

It will be noted that the results obtained for all cases approximate the reports of most observers, i.e., about 84 per cent of satisfactory results. It appears that skin sensitiveness, sex, type of extract, and route of administration do not seem to influence results. However, important and significant differences do appear as regards dosage and method of treatment (preseasonal or perennial). Here it will be noted that as the top dose is increased, the percentage of poor results is markedly decreased, so that the group receiving the top dose of 0.1 or more have a much lower percentage of poor results than the general average for the year, or the composite average for the five years. The cases receiving perennial treatment approximated the results obtained in the high dose group. It must be remembered, however, that the perennial cases were in the main in the high dose group. It is our opinion, therefore, that the apparent superiority of the perennially treated over the preseasonally treated cases is primarily one of high dosage.

This study supports the generally held view that better results are due to high dosage in the treatment of ragweed hay fever. From our findings, one can hardly explain the excellent results occasionally reported by others with low dosage therapy. Certainly, the reported failures in treatment with the perennial method are easily understood when associated with what seems to us to be extremely low dosage for this type of treatment. It would seem that the perennial method of treatment is the method of choice in those patients who can tolerate large doses at long intervals. Unfortunately, however, this method is not applicable to those individuals who, in the course of treatment, develop an intolerance to pollen extract. We have thought it wise to omit from consideration in this paper the results of a detailed investigation of the causes of failure in our small group of unsatisfactory results, including such factors as secondary sensitiveness to foods and inhalants, secondary paranasal sinus infections, poorly timed constitutional reactions, and other causes generally noted in such reports.

CONCLUSIONS

1. Results of specific therapy in late hay fever bear a definite relationship to the dosage of extract administered. In this study doses of ragweed extract of 0.1 or over yielded better results than the composite average for the whole group.

*Hereafter, in referring to dosage of pollen extract it will be understood that the figures refer to milligrams of total nitrogen.

2. An important factor in the apparent superiority of perennial over the preseasonal method of treatment is the high dosage employed in this form of treatment. Low dosage in the perennial form of treatment yields unsatisfactory results.

3. All other factors considered, namely, type of extract, sex of patient, degree of sensitiveness, and the route of injection, are shown to have no appreciable effect upon therapeutic results in hay fever.

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THE EFFECTS OF TOBACCO SMOKING ON HEALTH*

A Study of 2031 Medical Records

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DEBATES and controversies regarding the alleged harm to the human system from the use of tobacco still continue. The literature on the subject, extensive and often conflicting, varies in type from statistical studies on mortality, such as recently reported by Pearl,[1] to single experiments on individuals. Prejudice and emotion have so often entered into the problem that the question presents many complexities to the investigator who earnestly desires unbiased information on the relationship of tobacco consumption to the public health. A discussion of this subject, together with a review of the literature and a bibliography, has recently been published by Thienes and associates.[2]

*From Medical Examinations by the Life Extension Examiners, New York.

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In an attempt to learn what, if any, symptoms are produced in comparatively healthy ambulatory individuals by the use of tobacco, an unselected group of insurance policy holders were asked to fill out a questionnaire at the time of their periodic health examination. The questionnaire was designed to elicit their practices with reference to the use of tobacco, but contained no reference to physical signs or symptoms. This latter information was obtained independently from the physical examination and medical history which were recorded separately. Thus, by separating the time and method of obtaining the two kinds of information, it was hoped to avoid suggestion and bias.

Of 2,031 cases studied, 1,292, or 63.7 per cent, habitually used tobacco; 496, or 24.4 per cent, were nonusers; 104, or 5.1 per cent, used it only occasionally; and 139, or 6.8 per cent, were former users who had discontinued the practice.* No sex differentiation was made in this study. Chart 1 shows the trend in amount consumed by habitual users, though it must be admitted that the figures are only approximate. Weights were computed so that equivalent amounts might be included, regardless of method of use, whether through the smoking of cigarettes, cigars, or pipes. It will be noted that there was a tendency for increased consumption for each five-year period of use up to thirty years, when a sharp decline was noted.

Distribution of habitual smokers, according to duration of use, was as follows:

YEARS	NUMBER	PER CENT
1- 4	118	9.1
5- 9	193	14.9
10-14	243	18.8
15-19	247	19.1
20-24	191	14.8
25-29	121	9.4
30-34	87	6.7
35-39	40	3.1
40-44	33	2.6
45-49	12	0.9
50-54	7	0.5

Subjective symptoms and physical signs of smokers and nonsmokers are listed in Table I. Symptoms relating to the respiratory, circulatory, gastrointestinal, and nervous systems are definitely increased among smokers. These increases range from 50 per cent for palpitation of the heart to 300 per cent for cough. The increased tendency to dyspnea on exertion noted by the smokers gives point to the taboo on tobacco by many athletic directors, especially by the trainers of pugilists.

The relationship of tobacco smoking to gastric disturbances, especially to hyperacidity, is a frequent clinical observation. For this reason sufferers from gastric or duodenal ulcers are usually induced to refrain from tobacco. In this study, "heartburn" was increased by 100 per cent, and other digestive symptoms from 62 to 112 per cent.

That the two groups listed in Table I were fairly comparable is indicated by the average ages, which were nearly identical. There was no significant variation between the two groups in weight, pulse rate, or blood pressure. Tremors appeared to be diminished in the smoking group. The writers[3] have

*This does not represent the average practice of the population at large.

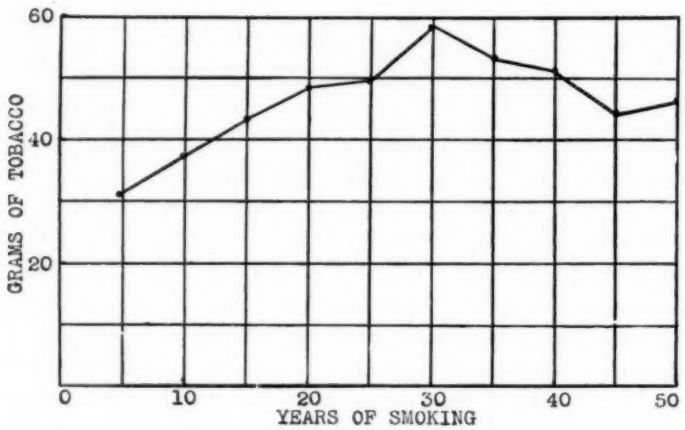

Chart 1.—Average amounts of tobacco consumed for each five-year period of use

Chart 2.—Reasons given for discontinuing the use of tobacco.

TABLE I

SYMPTOMS, FINDINGS, AND AVERAGES FOR SMOKING AND NONSMOKING GROUPS

SYMPTOMS AND FINDINGS	NONSMOKERS		SMOKERS		PERCENTAGE INCREASE OR DECREASE
	NUMBER	PER CENT	NUMBER	PER CENT	
Symptoms					
Respiratory system					
Irritation of nose and throat	12	2.4	82	6.4	+167
Cough	8	1.6	83	6.4	+300
Frequent colds	54	10.9	233	18.0	+ 65
Circulatory system					
Palpitation	14	2.8	54	4.2	+ 50
Precordial pain or "stress"	28	5.6	125	9.7	+ 73
Dyspnea on exertion	24	4.8	148	11.5	+140
Gastrointestinal system					
"Heartburn"	3	0.6	15	1.2	+100
Excessive gas	63	12.7	266	20.6	+ 62
Miscellaneous	38	7.7	211	16.3	+112
Nervous system					
"Nervousness"	19	3.8	86	6.7	+ 76
Findings					
Tremors	57	11.5	129	10.0	- 13
Premature heart beats	2	0.4	8	0.6	+ 50
Systolic heart murmurs	41	8.3	136	10.5	+ 27
Average age	36.8		37.2		+ 1
Average weight	153.7		154.0		+ 0.2
Average pulse	78.5		78.3		- 0.3
Average blood pressure	121/76		121/78		0/+3

noted, however, that there is a tendency for tremors to be increased if habitual users are too long deprived of tobacco, also that blood pressures tend to vary considerably during the actual process of smoking.

Reasons given for the discontinuance of tobacco by 139 individuals are graphed in Chart 2. Seventy-eight, or 56 per cent, in this group gave up smoking for reasons of health.

COMMENT

The complaints recorded are of especial significance because they were entirely spontaneous, written by the examinee on a history form, and not elicited by questioning. It should be emphasized that both groups were treated exactly alike and the findings therefore cannot be biased. We believe that the findings in this study indicate a trend in accordance with the recent report of Pearl, who found mortality markedly increased among heavy smokers. It is hoped that further studies will demonstrate more definitely the manner in which tobacco exerts its influence and the chief hazards attached to its usage.

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A DIRECT COMPARISON OF THE REACTIONS OF THE HUMAN SYSTEM TO TOBACCO SMOKE AND ADRENALIN*

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FOLLOWING the observations that the smoking of cigarettes produced a lowering of peripheral skin temperatures[1-3] and elevation of blood sugar,[4] it was considered of interest to make a direct comparison of the effects of adrenalin injection with those of tobacco smoking. Our procedure is here described.

PROCEDURE

Five normal habitual smokers in the postabsorptive state, after preliminary control observations, were studied for skin and oral temperature, blood pressure, and pulse rate changes, and for blood sugar and cholesterol fluctuations for one and one-half hours while cigarettes were being smoked. The skin temperature observations were made on a continuously recording apparatus by means of a thermocouple attached to the dorsum of the terminal phalanx of the finger. The blood pressures and pulse rates were taken at five-minute intervals; the oral temperatures and blood specimens for cholesterol and sugar at fifteen-minute intervals.

One week later the same subjects were treated in exactly the same way, except that, instead of the smoking of cigarettes, 1 c.c. of 1:1000 adrenalin solution was injected subcutaneously, following the control observations. Because of the rather large volume of individual observations and the tendency for wide fluctuations to occur, the results have been compiled as composite rather than as individual curves.

A comparison of the findings is expressed graphically in Charts 1-5.

FINDINGS

Chart 1 shows a remarkable parallelism in the pulse rates during tobacco smoking and after the injection of adrenalin solution. There was a decided increase which reached a peak at the end of about one-half hour and gradually diminished thereafter. The blood pressures generally showed a similar parallelism, but with striking fluctuations which were not altogether ironed out even in the composites. The most striking part of the blood pressure study was the lowering of the diastolic pressure after adrenalin,[4] an effect which would hardly be expected on theoretical grounds, since adrenalin produces a peripheral vasoconstriction. A compensatory mechanism probably accounts for this phenomenon. The diastolic pressure after cigarette smoking remained more constant at about the normal average values.

*From the Research Department of the Life Extension Institute, New York.
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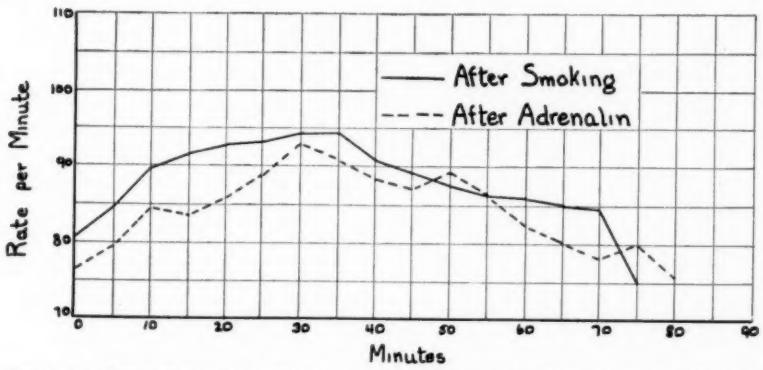

Chart 1.—Composite pulse rates after smoking and after adrenalin injection.

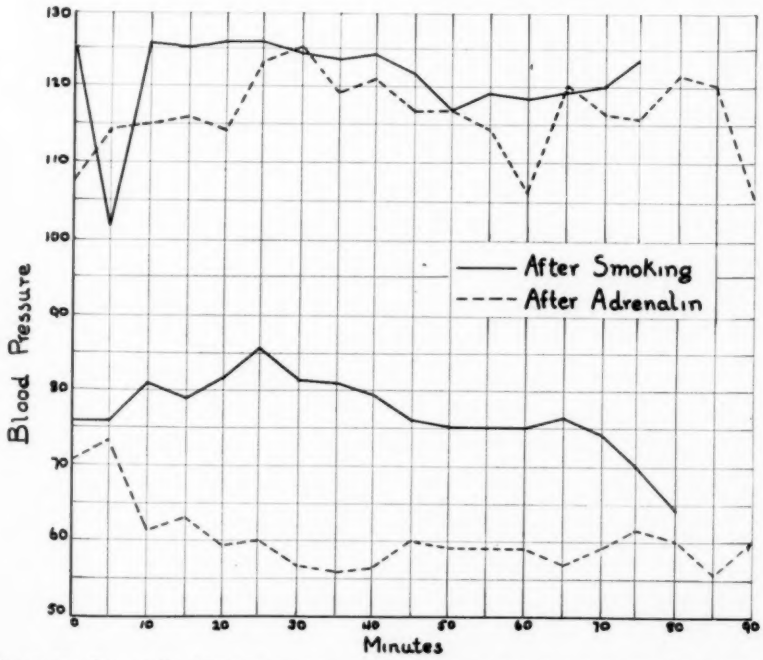

Chart 2.—Composite blood pressures after smoking and after adrenalin injection.

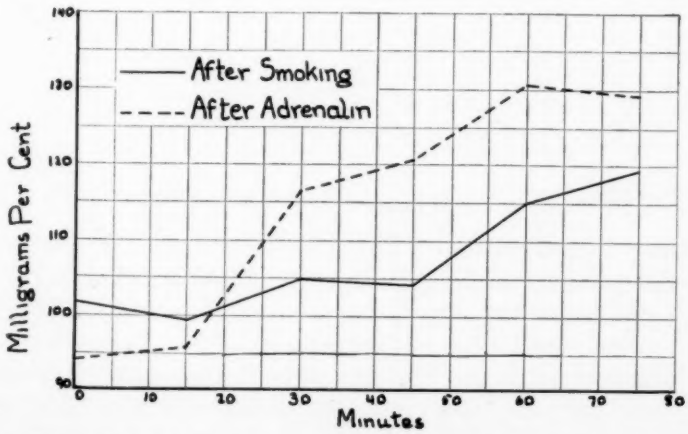

Chart. 3.—Composite blood sugar curves after smoking and after adrenalin injection.

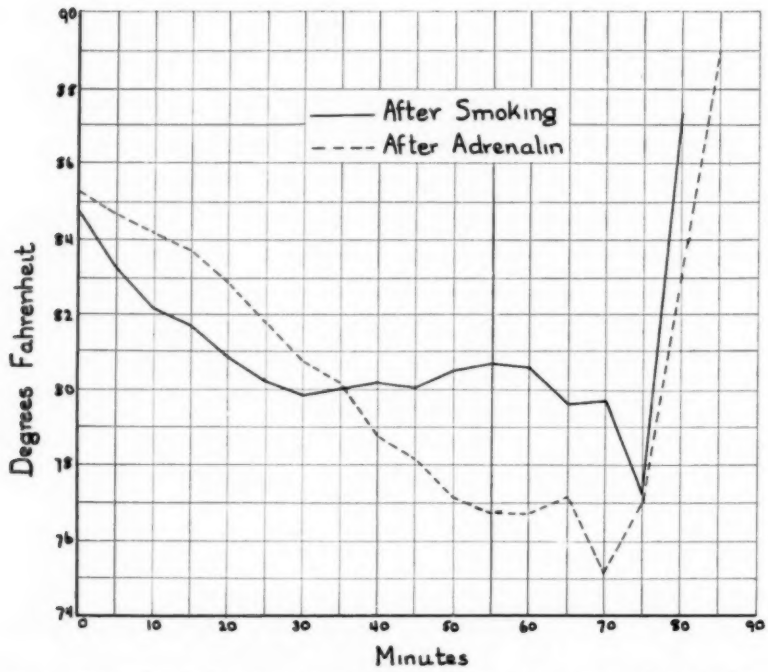

Chart 4.—Composite skin temperatures after smoking and after adrenalin injection.

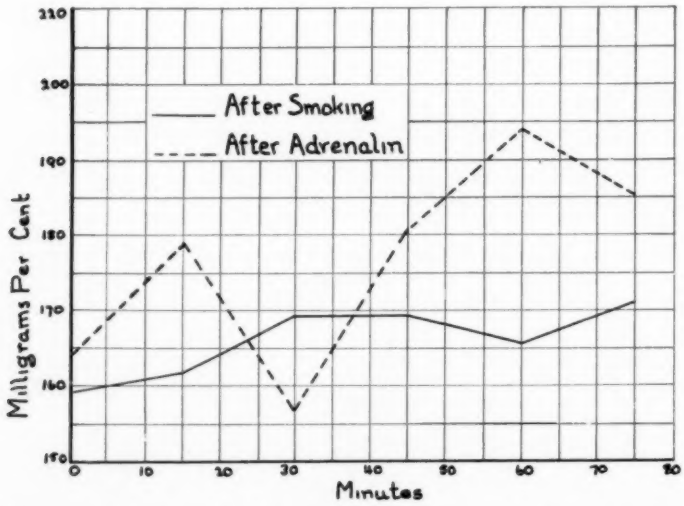

Chart 5.—Composite total blood cholesters after smoking and after adrenalin injection.

Confirming the observations of Haggard and Greenberg,[5] blood sugar values were seen to increase at fifteen-minute intervals from normal to an average upper value of 119 mg. during the seventy-five minutes of smoking. The results after adrenalin injection were similar but more pronounced. The composite curves of the skin temperatures during the two experiments were decidedly similar. During the smoking period the temperatures dropped from 84.6° F. to a low

point of 77.4° F. After adrenalin, the temperatures diminished from 85.3° F. to a low point of 75.2° F. After a period of about one and one-quarter hours, the temperatures during both experiments showed an abrupt increase to 87.4° F. and 89° F., respectively.

Total blood cholesterols were also studied in these experiments. It was noted that there was a slight tendency to an increase during tobacco smoking and a more marked increase after adrenalin. The adrenalin experiment, however, produced marked fluctuations.

COMMENT

Sollmon[6] states that blood pressure may be raised by nicotine, even in the absence of the vasomotor center, but not after the suprarenals have been excised. He attributes the blood pressure elevation to an increased output of epinephrine. Stewart and Rogoff[7] reported an increased output of epinephrine after nicotine administration, followed by a marked and enduring decrease. Rogoff attributes this to a stimulation of the adrenal medulla through the sympathetic nervous system.

In the foregoing experiments, we believe that the parallelism between the effects of injected adrenalin and smoking of tobacco is most significant. That the results were not altogether identical was to be expected because of the impossibility of adjusting the dose of adrenalin to correspond to the increment added to the blood by nicotine stimulation. Then too, absorption of injected adrenalin is an unpredictable and variable factor.

CONCLUSION

Our observations confirm the view that the characteristic effects upon pulse, blood pressure, peripheral skin temperature, and blood sugar of tobacco smoking can be explained by an increased output of epinephrine. This most probably is the result of a stimulating effect of nicotine upon the sympathetico-adrenal system.

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HISTOLOGY OF DEMODEX FOLLICULORUM*

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IN THE course of studies endeavoring to ascertain more efficient chemicals for the treatment of Demodex and Sarcoptic infection in man and animals, we obtained histologic sections of *Demodex folliculorum* which we are here presenting.

The mites exhibit a great variety of habits. They live principally on fluid nutriment which is obtained from living plants or animals or from decaying organic matter. The parasitic mites are of great interest on account of the wide variety of their habitats. Many like the ticks are external parasites of animals, feeding on the blood of their hosts; some like Sarcoptidae and Demodicidae, burrow in the skin of their hosts and cause severe itching and disease. Many species attack birds and feed on the scales and feathers, or even invade the lungs, air sacs, and hollow bones. Of still greater importance has been the discovery that many blood-sucking mites may serve as intermediate hosts of various pathogenic organisms of man and animals.

The Demodicoidea.—The Demodicoidea, the hair follicle mites, are a highly aberrant group of mites. They are parasitic in the hair follicles of mammals. They are elongate, the legs reduced to mere stumps, the abdomen vermiform, and the mouth parts modified, minute and fitted for piercing. The super-family contains but a single family, the Demodicidea and the one genus Demodex. The hair follicle mites of different animals are extremely difficult, if not impossible, to differentiate as distinct species.

Demodex folliculorum Simon is the hair follicle mite of man. It is abundant in some countries, but it is said to be rare in North Africa. It lies deep down in the hair follicles and the sebaceous glands. The entire life cycle is passed on the host, so that the infection gradually spreads.

Demodex canis Leydig, with which we are dealing here, attacks dogs and is cosmopolitan in distribution. It causes the follicular or red mange of the dog. The disease is serious. *D. cati* Megnin, the cat, *D. bovis* Stiles, cattle, *D. equi* Daillet, horses, and other species are formed on different mammals. Several workers have recorded *D. canis* parasitizing man. It would, however, appear quite probable that species commonly occurring in our domestic animals may attack man.[1]

Characteristics of Demodex folliculorum.—These wormlike mites attain a length of about 0.3 mm. and a width of 0.045 mm. They have lanceolate lyrate

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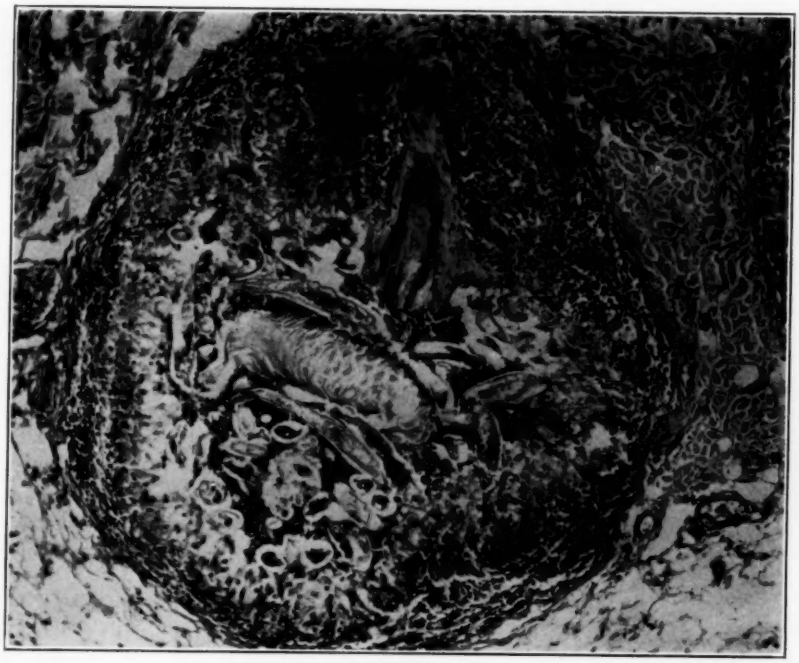

Fig. 1.—This section shows a whole lesion with the hair shaft in the center. The most prominent feature is the circular wall around the lesion, consisting of a rather heavy infiltration of round cells from without. The hair shaft in the center is cut tangentially and is in the process of autolysis. On top of the shaft a parasite is seen cut sagittally and below the shaft are two more parasites, cut the same way. In the surrounding tissue adjacent to the shaft and within the wall of the lesion many parasites are seen, most of them being cut transversely.

Fig. 2.—For details of the parasites this section is a high power enlargement of a small center part of Fig. 1. Three parasites (*a*, *b*, *c*) are showing structural details, especially *a*, having eight pairs of stumpy legs and the capitum showing clearly.

or horseshoe-shaped head, with shearlike masticatory apparatus, mobile proboscis, and triple-jointed maxillary antennae, an elongated cone-shaped abdomen, and eight triple-jointed stumpy feet attached to the thorax. The eggs are spindle-shaped. The larvae, when emerging from the egg, have the same general form as the fully equipped mites but have only six feet.

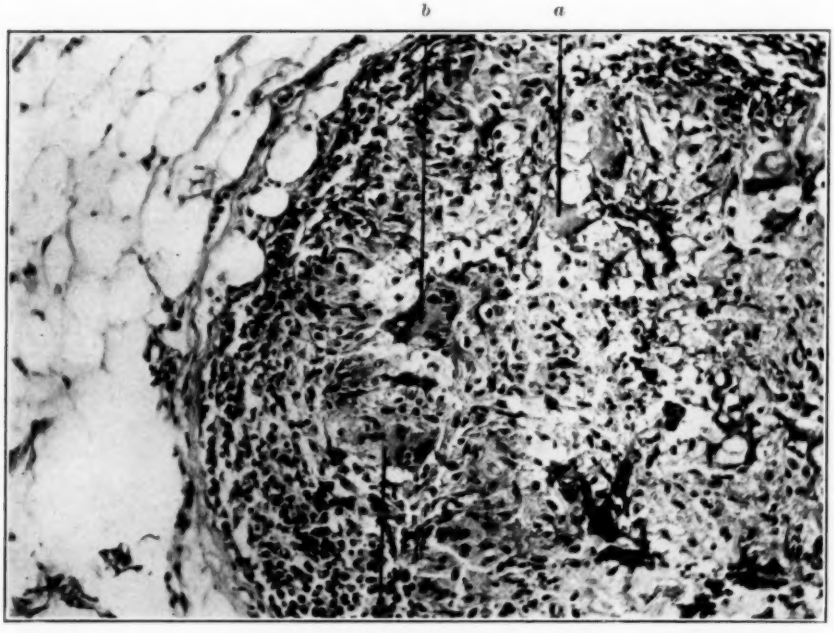

Fig. 3.—This section shows the isolation of the lesion by a heavy wall of connective tissue, infiltrated densely by round cells. Besides these pathologic changes this picture shows the formation of multinucleated giant cells (*a*, *b*, *c*) and the engulfing of a parasite (*a*) probably in its larval stage, i.e., active phagocytosis.

These parasites are found in the hair follicles and sebaceous glands, their head toward the bottom of the sac and the caudal end toward the external opening. Their number may be large in a sebaceous gland, even as many as 150 having been found there. They generally average from 30 to 50. The accompanying photomicrographs confirm this statement. The larvae and eggs, often in great numbers, lie beside the parasite. The irritation of the parasite causes the follicles and sebaceous glands to become dilated, and the subsequent atrophy of the hair papillae causes falling out and permanent loss of hair.

SUMMARY

The histologic sections of *Demodex folliculorum* in the epidermis of the dog are described, and the appearance of the parasites in situ is shown in the photomicrographs.

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THE RELATION BETWEEN SKIN SENSITIVITY, LIVER FUNCTION, LEUCOPENIC INDEX, AND TOXIC EFFECTS FROM CINCHOPHEN*

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FOLLOWING our original observation[1] that amidopyrine produced agranulocytosis in certain patients, an extensive series[2] of patients receiving this drug was studied carefully. The reproducibility of the agranulocytosis, which was confined to 1 per cent of the patients, suggested that it was caused by hypersensitivity to the drug, but skin sensitivity tests with aqueous solutions gave essentially negative results. Horsfall[3] demonstrated that, while aqueous solutions of formaldehyde gave negative skin tests, a combination of blood serum with formaldehyde gave positive reactions. This suggested an explanation for our failure to obtain positive skin tests with aqueous solutions of amidopyrine. Damashek,[4] following the principle of Horsfall and working with patients who had had agranulocytosis and in whom an etiologic relationship had been established, found that, while aqueous solutions of amidopyrine did not give positive skin tests or produce constitutional symptoms, as little as 1/6 gr. of amidopyrine mixed with blood serum and injected intradermally gave positive reactions, and precipitated an attack of the disease in certain cases.

In view of our own experience,[1, 2] this was regarded as conclusive evidence that the agranulocytosis in these patients was due to drug hypersensitivity. It suggested that, since the toxic symptoms from amidopyrine were probably due to allergic phenomena, toxic symptoms from other drugs might also be due to allergy. Damashek[4] did not make intradermal tests on a large number of other patients and confirm the tests by oral administration of amidopyrine. It was considered advisable, therefore, to make further study along these lines, using a large series of patients and a drug which is more frequently toxic than pyramidon, viz., phenylcinchoninic acid (cinchophen), to determine: (1) the relation between skin sensitivity, liver dysfunction, and the development of toxic symptoms; and (2) whether skin tests were of value for eliminating patients who are likely to develop toxicity from cinchophen. The leucopenic index was determined in 20 cases.

MATERIALS AND METHODS

The patients were selected without discrimination on admission to the clinic. Skin sensitization tests and liver function tests were made on all patients before cinchophen was administered. Great care was taken to eliminate experimental

*From the Arthritis Clinic of the New York Polyclinic Medical School and Hospital, New York.
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TABLE I

RELATION BETWEEN INTRADERMAL SENSITIVITY, LIVER DYSFUNCTION, AND CINCHOPHEN TOXICITY

PATIENT	INTRADERMAL SENSITIVITY DILUTIONS			LIVER DYSFUNCTION	TOXIC SYMPTOMS AFTER TAKING CINCHOPHEN							
	I	II	III		NAUSEA	URTICARIA	ICTERUS TINGE	RASH	ITCH	DIZZINESS	NERVOUSNESS	OTHER SYMPTOMS
Patients With Toxic Symptoms After Taking Cinchophen												
F. F.	0	++	++	+	+					+	+	
O. K.	0	+	++	+	+					+	+	
M. R.	+	++	+	+	+					+		
C. O.	++	++	++	+	+	+						
J. G.	+	++	+++	+	+							Depressed
S. M.	++	++	0	+	+		+	+				
R. C.	+	++	+++	+	+					+	+	Insomnia
F. B.	+	+	+	+	+					+	+	
F. P.	+	++	+	+	+							Vomiting
C. G.	0	++	+	+		+						
H. S.	+	+	+	+				+	+			
M. D.	++	++	++	+						+	+	
E. D.	++	++	++	+						+	+	Irritable
S. E.	+	++	+++	+					+		+	
S. C.	+	++	+++	+				+	+			Restlessness
F. W.	+	++	+++	+						+	+	
M. M.	0	++	+++	+						+	+	
T. W.	0	++	++	+				+	+		+	
A. S.	0	0	0	+								Diarrhea*
A. N.	0	+	+	+				+	+			
C. R.	++	++	++	0	+							
M. S.	+	++	+	0	+			+	+			
P. S.	+	++	+++	0	+			+				
A. P.	++	+	+	0		+						
M. H.	0	+	+++	0	+					+		
F. B.	0	0	0	0		+					+	
N. D.	0	0	0	0				+	+*			
M. G.	0	0	0	0				+	+			
M. M.	0	+	0	0			+	+				
J. T.	0	0	+	0		+						
Patients Having No Toxic Symptoms After Taking Cinchophen												
W. P.	0	+++	++	+								
S. C.	++	+	+	+								
C. S.	0	++	++	+								
C. C.	+	++	+++	0								
V. L.	+	++	+++	0								
L. O.	+	+	+	0								
E. T.	0	0	0	+								
J. B.	0	+	+	+								
J. P.	0	+	+	+								
M. M.	0	+	+	+								
M. R.	0	0	0	0								
E. W.	0	0	0	0								
L. B.	0	+	+	0								
M. C.	0	0	0	0								
T. R.	0	0	0	0								
A. G.	0	0	0	0								
R. L.	0	0	0	0								
T. M.	0	0	0	0								
C. T.	0	0	0	0								
O. K.	0	+	+	0								

*Severe.

error, which is apt to occur in this type of work, and any suspicious findings were checked. Cinchophen gr. viiss t.i.d. was given for four weeks, provided toxicity did not develop.

A 5 per cent solution of sodium cinchophen was combined with blood serum as follows: 1.0 c.c., 2.0 c.c. and 3.0 c.c. quantities of the cinchophen solution were mixed with 5.0 c.c. of blood serum, labeled dilutions No. I, II, and III, respectively, and kept in the refrigerator four days. As a control, 5.0 c.c. of blood serum mixed with 3.0 c.c. of normal saline also was placed in the refrigerator four days. The injections consisted of 0.05 c.c. intradermally on the forearm. Readings were taken in thirty minutes because the reactions reached a maximum by this time. Only those reacting 2+ or more with any of the dilutions, or 1+ with all dilutions, were considered positive. An occasional patient who gave a positive reaction with the control serum was not included in the positive group.

The azorubin S, hippuric acid, and bilirubin excretion tests were used to determine liver function since we showed[5] that they were the most reliable in chronic arthritis. A positive result with one or more of these tests was considered evidence of liver dysfunction.

The technique and interpretation of the leucopenic index were described by Vaughan.[6] The mean fasting white blood cell count was determined. One tablet (gr. viiss) of cinchophen was crushed and given with a small amount of water to promote rapid absorption. Blood counts were taken after fifteen, thirty, forty-five, sixty, and ninety minutes, and again after twenty-four hours.

The following symptoms were considered evidence of cinchophen intoxication: urticaria, scarlatiniform rash, angioneurotic edema, nausea and vomiting; jaundice, distention with abdominal pain, and loss of appetite; nervousness associated with depression, hematuria, and diarrhea.

Analysis of 50 Cases.—Of the 50 patients studied, 30 (60 per cent) developed signs of toxicity attributed to cinchophen. Twenty-three (77 per cent) of this group gave positive skin reactions to cinchophen.

Twenty-nine (58 per cent) of the entire group gave positive skin reactions, and 23 of these (79 per cent) developed toxic symptoms. Seven (33 per cent) of the 21 patients with negative skin tests developed toxic symptoms.

In 6 patients, all with positive skin tests, the skin test dose alone produced toxic symptoms. Five of them also developed toxic symptoms after oral administration.

Relation Between Liver Function, Skin Tests, and Cinchophen Toxicity.—Liver function tests were made on all 50 patients before cinchophen was administered. Fifteen (71 per cent) of those with negative skin reactions had normal liver function tests, while 73 per cent of patients with positive skin tests had liver dysfunction. Sixty-seven per cent of the patients who developed toxicity showed evidence of liver dysfunction as compared with only 35 per cent of those who did not develop toxic symptoms. Of the 30 who developed toxicity, 60 per cent gave both positive skin and liver function tests, while 50 per cent of the 20 who did not develop toxicity gave both negative skin and liver function tests. The relationships confirm the opinion of previous investigators that liver damage may predispose to toxicity from cinchophen.

Leucopenic Index.—Vaughan[6] reported that, in certain patients with food allergy, there was a drop in the total leucocyte count after the ingestion of food to which they were sensitive. The leucopenic index, which has been used as a test for sensitivity to different agents, was done on 20 patients who had previously shown toxic symptoms after taking cinchophen, and 9 (45 per cent) of them were positive. The leucopenic index, therefore, did not compare favorably with the skin test, which gave positive reactions in 77 per cent of the patients who developed toxicity.

DISCUSSION

One hesitates to propose a diagnostic procedure based on skin tests alone because they are prone to considerable error. Tests were repeated on the first 25 patients to determine the reproducibility of the tests and similar results were obtained in 21 (84 per cent). Frequent rechecking of other patients also gave similar results. However, the results with each dilution were not always constant. The intensity of the skin reactions was not proportional to the amount of drug injected. In some instances the reaction from dilution No. I, containing the least amount of cinchophen, was more intense than those from dilutions No. II and III. In other instances, the reactions varied according to the concentration of cinchophen-serum mixture. These findings suggest that the results can be used qualitatively but not quantitatively, and that the experimental error can be minimized by using different amounts of test substance and by rechecking doubtful results.

There did not appear to be any relationship between the intensity of the skin reactions, the interval before toxicity developed, and the severity of the toxic symptoms.

CONCLUSIONS

1. Seventy-nine per cent of the patients who showed positive intradermal tests with the cinchophen-serum mixture developed toxic symptoms after cinchophen was administered by mouth, while only 33 per cent of the patients giving negative tests developed toxic symptoms.

2. Seventy-one per cent of the patients with negative skin reactions had normal liver function tests, while 73 per cent of those with positive skin tests had liver dysfunction.

3. Liver dysfunction was present before treatment in 67 per cent of patients who developed toxic symptoms after taking cinchophen by mouth, as compared with 35 per cent in those who did not develop toxic symptoms.

4. The leucopenic index did not prove to be as valuable as the intradermal tests for determining the likelihood of patients developing toxicity from cinchophen.

5. The results of this work suggest that intradermal tests with drug-serum mixtures may be of value in determining drug toxicity and should receive further study.

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COMPARISON OF INTRADERMAL TESTS WITH AGGLUTINABILITY AND CERTAIN IN VITRO TESTS OF STREPTOCOCCI, STAPHYLOCOCCI, MICROCOCCUS CATARRHALIS, AND COLON BACILLI ISOLATED FROM PERSONS SUSPECTED OF HAVING CHRONIC INFECTION*

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INTRADERMAL tests have been used widely, not only for the differentiation of "focal infection" bacteria, but also in the selection of bacteria for the preparation of autogenous vaccines. The literature is too voluminous to be reviewed here. The following papers, however, have a special bearing on the subject.

Feinberg[1] presented an excellent critique of the limitation of skin tests in allergy.

Steinberg and Wiltsie[2] obtained reactions with *B. coli* toxic filtrate in all 60 normal children and all 40 normal adults. These results are similar to those obtained by workers in other allergens. For example, Grow and Herman[3] obtained 55.5 per cent positive results with common allergens in a group of 150 normal individuals.

Inconclusive results have been reported also in persons with different diseases. Steinberg and Wiltsie[2] found that the skin reaction to *B. coli* was not related to the presence of infection. Four of 11 patients with pyelitis did not react to *B. coli* toxic filtrate, and 5 of 11 did not react to *B. coli* vaccine. They concluded that "under the conditions of these experiments, the skin reaction for the determination of the presence of colon bacillus infection is of uncertain value." Solis-Cohen[4] concluded that "There probably is no relationship between hypersensitiveness in the host to the exogenous and endogenous toxins of a given organism and the pathogenicity of such organism for that host." His conclusion that intracutaneous tests are unreliable for selecting bacteria for vaccines seems to have been shared by most recent

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writers on the subject, although many of them, like Moore,[5] admitted the fallacy of the tests but concluded that they are "a very important factor as an aid to diagnosis of allergic diseases."

Much of the published data is difficult to interpret because of errors in bacteriologic diagnosis or the use of indefinite bacteriologic names. For example, one writer stated that 105 of his 133 strains of hemolytic streptococci were isolated from the gastrointestinal tract. It is more likely that many of these cultures were hemolytic enterococci, which are mostly nonpathogenic and possess properties distinct from other hemolytic streptococci. Other workers refer to any intestinal streptococcus as *Streptococcus fecalis* or to small, hemolytic surface colonies as hemolytic streptococci. Many of the latter may be alpha hemolytic or alpha prime streptococci, or may not even be streptococci.

Many of the published reports concerned work which was inadequately controlled. One writer reported using an average of only 3.6 tests per patient and 2.9 tests per control case.

Finally, the difference between the proportion of positive reactions in patients and in the controls does not show a marked contrast. In comparing patients with irritable colon with normal individuals, Mateer and Baltz[6] obtained diameters of 4.4 and 3.0 cm., respectively, with *B. coli communis;* 4.5 and 2.7 cm. with *B. coli communior;* 2.7 and 1.0 cm. with nonhemolytic streptococci; and 2.4 and 1.0 cm. with *Staphylococcus aureus.* The tests with *B. coli communis* were positive in 95 and 65 per cent, with *B. coli communior* in 96 and 69 per cent, with nonhemolytic streptococci in 62 and 21 per cent, and with *Staphylococcus aureus* in 49 and 50 per cent of patients and controls, respectively. Mateer and co-workers[8] obtained similar results.

Short, Dienes, and Bauer[7] maintained that variations in the skin reactions may be explained by differing irritability of the patients' skins, natural toxicity of the bacterial species, or possibly by a sensitization to certain bacterial groups.

Mateer and Baltz[6] claimed that the reaction decreased after immunization with the specific vaccine. However, their report indicates that the average reactions to *B. coli* were reduced only from 4.7 to 3.1 cm. in 12 patients immunized with *B. coli.* Steinberg and Wiltsie[2] found an average area of 9.7 by 6.5 cm. before, and 3.9 by 2.3 cm. after immunization with *B. coli.*

Since certain in vitro tests have been shown to give results parallel with certain pathogenic properties of the cultures,[9-15] it was thought that these in vitro tests might be useful in comparing the results of intradermal tests. Agglutination tests were used for comparison also. A series of 305 smooth cultures was tested.

For the intradermal tests the bacteria were suspended in 1.0 per cent phenol in normal saline to make concentrations of 1 billion per c.c., and 0.05 c.c. was injected intradermally on the forearm. The results were read the following day.

The technique of the agglutination tests is described elsewhere.[16]

Pigment, hemolysis, and coagulase tests[9] were used as in vitro tests of staphylococci; resistance to the bactericidal action of fresh, diluted, defibri-

nated guinea pig blood[10, 11, 15] was used for streptococci; the electrophoretic migration velocity[12, 13] was used for the colon group; and the crystal violet agar reaction[14] was used for *M. catarrhalis*.

TABLE I

RELATION BETWEEN INTRADERMAL TESTS, AGGLUTINABILITY AND IN VITRO TESTS OF PROBABLE PATHOGENICITY OF CULTURES ISOLATED FROM PERSONS SUSPECTED OF HAVING CHRONIC INFECTION

ORGANISM	CULTURES GIVING POSITIVE INTRADERMAL TESTS			CULTURES GIVING NEGATIVE INTRADERMAL TESTS			PER CENT AGREEMENT BETWEEN SKIN TESTS AND IN VITRO TESTS	PER CENT AGREEMENT BETWEEN SKIN TESTS AND AGGLUTINABILITY
	NUMBER TESTED	IN VITRO POSITIVE	AGGLUTINABLE	NUMBER TESTED	IN VITRO NEGATIVE	INAGGLUTINABLE		
B. coli	19	2	5	11	10	10	40	50
A. aerogenes	9	5	2	2	0	2	45	36
Paracoli	4	0	0	4	4	2	50	25
Enterococci	4		2	19		19		91
Strep., gamma	3	0	1	14	12	14	70	88
Strep., alpha	11	5	1	105	58	65	54	57
Strep., beta	4	4	0	3	0	2	57	28
M. catarrhalis	1	0	1	5	5	5	83	100
Staph. albus	7	4	1	57	50	43	84	69
Staph. aureus	8	8	5	15	11	11	87	70

The results of the comparative tests are listed in Table I. There was agreement between intradermal and in vitro tests in 83 per cent of *M. catarrhalis* cultures, 84 to 87 per cent of staphylococci, and in 70 per cent of gamma type streptococci. Other groups showed agreement in less than 57 per cent of the tests.

There was agreement between intradermal tests and agglutinability in 91 per cent of enterococci, 88 per cent of gamma type streptococci, 100 per cent of *Micrococcus catarrhalis*, and 69 to 70 per cent of staphylococci. Other groups showed agreement in less than 57 per cent of tests.

CONCLUSIONS

Intradermal tests of bacteria isolated from patients suspected of having chronic infection were compared with (1) agglutination reactions of the strains using the serum of the person from whom the cultures were obtained and (2) in vitro tests which had been shown previously to have been parallel with certain pathogenic properties of the cultures.

The intradermal tests showed agreement with either agglutinability or the in vitro tests in more than 70 per cent of *Micrococcus catarrhalis*, staphylococci, gamma type streptococci, and enterococci.

There was less than 57 per cent agreement with either test in alpha and beta type streptococci, *B. coli*, *Aerobacter aerogenes*, and paracoli.

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PRELIMINARY REPORT ON THE USE OF SULFANILAMIDE*

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AFTER making rather tentative use of sulfanilamide in a few cases of acute infections, and after noting its often spectacular effect, we studied the drug more intensively, in order to determine, if possible, the mechanism by which it acts, and the extent of its clinical possibilities.

This paper concerns the way sulfanilamide produces its therapeutic effect and the results of its use in a variety of acute and chronic infections. A drug may assist a patient in his battle against invading bacteria: (1) by making the patient's tissues an unsuitable culture medium, bacteriostasis; (2) by killing the bacteria, a bactericidal or bacteriolytic effect; (3) by increasing the number of leucocytes, and hence presumably the number of phagocytes; (4) by increasing the effectiveness of the existing phagocytes, enabling them to engulf and inactivate or kill a larger number of bacteria by increasing the opsonins in the patient's serum; (5) by inactivating the toxin produced by the bacteria, an antitoxic effect; (6) by making the tissues of the patient less sensitive or less susceptible to injury by toxin; (7) by causing the organism to assume less virulent forms, dissociation.

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In this paper we report observations bearing definitely on the first, third, fourth, fifth, and seventh possibilities. The studies were made simultaneously on each patient before, during, and after treatment with prontylin (p-aminophenylsulfonamide) and with "Prontosil (disodium 4-sulfamidophenyl-2-azo-7-acetyl-amino-1-hydroxynaphthalene 3, 6 disulfonate) 2.5 per cent solution." These substances were furnished by the Department of Medical Research of the Winthrop Chemical Co. In our work the clinical effect was checked as far as possible bacteriologically.

Phagocytosis.—Sulfanilamide induced a high degree of phagocytosis in experimental hemolytic streptococcic peritonitis in mice according to Long and Bliss.[1]

Tunnicliff's method[2] of measuring phagocytosis and titrating opsonins was used in most instances. Occasionally it was necessary to use the older method with the patient's serum and indifferent leucocytes. The opsonins for the patient's own bacteria were titrated whenever the invaders could be isolated. In each instance the opsonins for strains of greening streptococci recently isolated from the blood of a patient with acute arthritis and for a Type I pneumococcus (American Type Culture) were also determined. The opsonic index was measured before, during, and after treatment with prontylin or prontosil. The most positive increase in opsonic titer, after the use of sulfanilamide, was observed in pneumonia. This increase of opsonic ability was coincident with improvement of the patient. It may be only a natural accompaniment of convalescence rather than a drug effect. Otherwise there was no suggestion that the drug affected phagocytosis in patients.

Leucocytosis.—Daily leucocyte counts on patients and on three normal individuals before, during, and after treatment with prontylin and prontosil showed no variation other than the usual daily fluctuation.

Inactivation of Bacterial Toxin.—Evidence is lacking to show any antitoxic tendency on the part of sulfanilamide or its compounds.

Inhibition of Bacterial Growth.—Prontylin in vitro in a dilution of 1:100,000 markedly inhibited the growth of streptococci. *B. coli* from a stock culture showed no growth at 1:200,000, but a recently isolated strain grew well even in an 8 per cent solution. We have been able to grow streptococci only in dilutions higher than 1:20,000 and even then the growth was very sparse. According to Marshall, Emerson, and Cutting,[3] concentrations between 1:5,000 and 1:10,000 can be maintained in the blood of those treated. From the marked effect upon bacterial growth encountered in our in vitro experiments, this would seem to be one of its important modes of action in infection.

Dissociation.—Alterations of bacteria, chemical, antigenic, cultural, and morphologic, may be anticipated from a drug that so radically lessens the growth of bacteria in vitro. These changes in the bacteria due to growth in an unfavorable environment come under the heading of dissociation. They may, at least in part, explain the favorable effect of sulfanilamide in disease. Such dissociation is suggested by one case in which green streptococci were isolated from the blood of a patient. After two weeks of intensive treatment with prontylin and prontosil, the blood flora had changed to indifferent streptococci. After one more week of treatment, cultures of the blood yielded no growth.

Another instance of this was found in a pneumonia patient. A green streptococcus was isolated in the first culture. After three days of prontylin, an indifferent organism was obtained, simultaneously with clinical improvement.

This report is concerned chiefly with the effect of sulfanilamide in pneumonia and arthritis. Patients with undulant fever, endocarditis, pneumococcus meningitis, and chronic cholangeitis are also reported.

CLINICAL COURSE OF TREATED CASES

Undulant Fever.—The first patient treated for undulant fever was a 31-year-old male who had handled raw beef and pork for several years. For three weeks before admission to Cook County Hospital he had had generalized aching, chills, and fever. These symptoms persisted in the hospital, his fever rising to 103° every afternoon. The only significant abnormality was an enlarged spleen. The leucocyte count varied between 7,000 and 8,000. The Widal test was negative, as were also the cultures of blood and urine.

At the time of admission, the serum was found to agglutinate both the porcine and bovine types of *B. melitensis* through a dilution of 1:320 on the slide and above 1:1280 in the test tube. Semi-weekly injections of both forms of *B. melitensis* had brought no improvement after three weeks. For three more weeks the patient had only supportive treatment. Then he was given sulfanilamide, 40 grains for one day, and 30 grains daily thereafter for one week. The temperature was normal in forty-eight hours and did not rise again during a month of convalescence. He has had no recurrence after one year.

The second patient was a 69-year-old white female. She had been ill for two months with intermittent attacks of fever, vague rheumatic pains, and marked loss of weight and strength. Her hemoglobin was 50 per cent, red blood cells 2,500,000, and leucocytes 3,000. All tests for malaria and intestinal parasites were negative. Blood culture, however, was positive for *B. melitensis*, and her serum agglutinated this organism in a dilution of 1:15,000. When she became comatose a few days later, she was given a blood transfusion and 30 grains daily of prontylin. After forty-eight hours, she had a normal temperature and made an uneventful convalescence. She has remained well in the nine-month interval.

Berger and Schnetz[4] have reported recovery from Malta fever following administration of prontylin.

Pneumococcic Infections.—Nine pneumonia patients were given sulfanilamide in the Cook County Hospital. The mortality there is high because the patients are usually aged, alcoholic, or poorly nourished. During this year the mortality in the Cook County Hospital was about 50 per cent. There was no choice of patients treated with sulfanilamide. The patients were given 45 grains of sulfanilamide orally every twenty-four hours, and 20 c.c. of "prontosil soluble" intramuscularly every twelve hours. Two patients died. One of these, moribund on admission, lived only thirty-six hours.

The other patient had a bacteriemia. Pneumococci in the sputum could not be typed by any of the available sera. He was very much improved, only to die suddenly during his convalescence. He was a marked alcoholic. During his treatment the blood organisms changed from greening cocci to indifferent bacilli, either under the influence of sulfanilamide or as a natural accompaniment of convalescence.

Of the 7 (70 per cent) who fully recovered, 2 had a bacteriemia.

The opsonins as measured by Tunnicliff's method increased sharply during the administration of sulfanilamide, either the result of the drug or part of a natural and expected increase in antibodies during the progress of an infection.

No immediate effect was seen which could be ascribed to a definite, prompt result of the drug in pneumonia.

A robust male of 45 years was given 10 c.c. injections of prontosil every eight hours for four injections, thirty-six hours after the onset of pneumococcic meningitis. The meningeal inflammation followed an acute otitis. He was in deep delirium when first seen. His spinal fluid was turbid and contained cells and innumerable pneumococci. He died sixty hours after the onset of his symptoms, without showing any good or bad effect from sulfanilamide administration.

Arthritis.—Of great interest were the results in arthritis. In this group we treated only patients whom we could bacteriologically control.

One patient, a woman, has rheumatoid arthritis. Her elbows, wrists, knees, and fingers have been swollen and tender intermittently in long periods for five years. The joint symptoms are worse with occasional bouts of chills and fever. Repeated examinations for malaria and undulant fever (including skin tests with Brucellin) have always been negative. Green streptococci have been isolated from her blood three times. Cultures of her colon taken according to our technique[5] have repeatedly yielded from 50 to 98 per cent green streptococci in spite of repeated implantations and feedings of acidophilus bacilli, and of rapidly growing colon bacilli (''Mutaflor'' Nissl). Colonic lavage improved her for a long time. In a flare-up with an average temperature of 100° F. and swollen knees, administration of 10 grains of sulfanilamide four times a day removed all signs of joint trouble and restored the temperature to normal within thirty-six hours. It also changed the colonic flora from almost a pure culture of green streptococci to apparently complete absence of streptococococci with a homogeneous growth of colon bacilli. The sulfanilamide was continued in doses of 5 grains three times daily for two weeks. Following this her colon remained relatively free of streptococci for three weeks. One month after discontinuing the drug the streptococcus count had risen to 50 per cent, and one week later the patient returned with an exacerbation of her joint disease.

Another woman of 59 years had had rheumatoid arthritis for years. Like the previous patient, all the usual foci had been removed. Green streptococci made up 70 to 90 per cent of her colonic flora at all times. She had a tendency to loose stools and abdominal distress. With these bowel upsets her joint symptoms were aggravated. Seven days after a course of 40 grains daily of sulfanilamide the streptococci had been completely eliminated from the colon wall. Her joints were somewhat less painful, but the drug induced a diarrhea controlled only by stopping it.

A woman of 72 years had had rheumatoid arthritis for eleven years. It had progressed to the point of marked disability. From one of her swollen knees turbid fluid was aspirated containing pleomorphic greening streptococci in pure culture. The culture of the blood was sterile. She took 30 grains of sulfanilamide daily for one week, and then 15 grains daily. Sixteen days after starting the drug the culture of the knee was sterile, although a smear of the synovial fluid showed degenerated, poorly staining streptococcus forms. The knee joint has remained sterile. The arthritis has been very much relieved and the patient's general health is much improved. The sedimentation reading, 45 per cent in one hour at the onset of treatment, fell within four weeks to 18 per cent. She gained weight and strength. No other treatment was employed during the period of sulfanilamide administration.

A woman of 34 years, with active tuberculosis of the lungs and cervix, complained of pain in the joints. Blood culture yielded indifferent streptococci. Following three weeks of sulfanilamide, the blood culture was negative and has remained so. Her arthralgia and tuberculosis were unaffected.

Cholangeitis.—A woman of 42 years had suffered a complete stenosis of her common bile duct during or following cholecystectomy. A choledochogastric fistula developed spontaneously. The whole upper abdomen became a mass of dense adhesions discouraging further surgery. The woman felt ill. She had enough hyperbilirubinemia, without visible jaundice, to produce pruritus. She complained of constant pain in the upper right abdomen and in the back. This sense of pressure increased during the day to become severe each afternoon with a chill and fever of 102° F. The paroxysm would be followed by profuse sweating. After carefully excluding malaria (by searching for malaria and administering quinine up to tolerance), undulant fever, gross hepatic abscess, and pyelocystitis, the attacks were explained as Charcot fever due to obstruction of the biliary passages with probably low-grade infection of the

biliary tract, type undetermined. Cholagogues and duodenal drainage (Meltzer-Lyon technique) were without effect. Competent surgeons twice found the upper abdomen a mass of dense adhesions and located a fistula between the hepatic ducts and the stomach (choledochogastric). The common duct could not be identified. The abdomen was closed without attempting the contemplated plastic surgery on the biliary ducts. Fever and chills continued. Administration of sulfanilamide 30 grains daily for three days resulted in complete disappearance of all symptoms. Her condition remained good during the medication and for two weeks after discontinuance of the drug. Recurrences were promptly checked by resuming the sulfanilamide in full dosage and eliminated by continuing the drug in daily doses of 1 gm.

Endocarditis.—Four patients with endocarditis received sulfanilamide. The course of two of these patients was apparently unaffected by the sulfanilamide. They died of cerebral embolism. Fever was eliminated from a third patient who later died of hemorrhage from a benign gastric polyp.

The other patient was 43 years old. He had had fever for ten weeks. He was very anemic, had petechiae and an enlarged spleen. He also had an enlarged heart with a systolic murmur. Greening streptococci were isolated from the blood. He ran a septic course for four weeks, becoming weaker daily. He became and remained afebrile after 60 grains of sulfanilamide. The drug was continued for three weeks. His blood became sterile and has remained so. After several weeks of observation, he left the hospital. He has returned since and successfully passed through a pneumonia. He remains well.

Unfavorable Effects.—Cyanosis attributable to the use of sulfanilamide has been observed in one instance. In this patient cessation of the drug "prontylin" and its substitution by oral "neoprontosil" in the same dosage resulted in freedom from the cyanosis. Complete blood counts, repeated at intervals of one to three days during the period of heaviest drug administration and weekly thereafter, have shown no changes not explained by the presence of infection. Frequent repeated urinalysis has never shown albumin, red blood cells, or casts suggesting renal irritation, nor detectable urobilinogen indicating liver injury.

Gastrointestinal irritation as manifested by nausea, burning, or diarrhea, has been the most frequent and most troublesome adverse effect.

SUMMARY AND CONCLUSIONS

Appreciating that too few patients have been observed, it is suggested that sulfanilamide may cure some patients of undulant fever.

Some patients subacutely or chronically infected by *Streptococcus viridans*, with arthritis or endocarditis, have been helped by the drug. Most interesting has been its effect upon the streptococci on the colonic mucosa.

Untoward effects have been limited to irritation of the gastrointestinal tract.

Bacteriostasis or limitation of bacterial growth and resultant limitation of injurious bacterial products as toxins, seems to us to be one probable mechanism of benefit by sulfanilamide in disease.

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A STUDY OF CALCIUM AND PHOSPHORUS IN CEREBROSPINAL FLUID*

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THE literature of cerebrospinal fluid calcium and phosphorus has been reviewed by Levinson and Katzenellenbogen.[1] The latter concluded that although the calcium content of the cerebrospinal fluid has been reported to be elevated in meningitis and some other diseases, the number of instances of increased calcium was too small to be of diagnostic significance. The most complete work on phosphorus has been that of Cohen[2] who found the phosphorus content to be definitely elevated in tuberculosis and meningococcic meningitis, but not in acute epidemic encephalitis. This led Cohen to consider that the cerebrospinal fluid phosphorus may be of diagnostic importance in diseases of the nervous system. On the whole, the relationships between the calcium and phosphorus contents of the cerebrospinal fluid and the condition of the central nervous system are far from clearly defined. We found very few references to calcium and phosphorus in hydrocephalus and brain tumor. Our results may be of interest, therefore, since they indicate increased calcium and phosphorus values in tuberculous and purulent meningitis, hydrocephalus, and brain tumor.

Calcium determinations were made on 92 specimens of the cerebrospinal fluid; phosphorus determinations were made on 76 specimens. The specimens of cerebrospinal fluid were obtained from hospital patients, most of whom were children, at times when spinal punctures were necessary for diagnostic or therapeutic purposes. Lumbar punctures were made, except where otherwise indicated. Calcium was determined by the Clark and Collip modification of the Kramer-Tisdall method.[3] Phosphorus was determined by the method of Bodansky,[4] except that in some cases 2 c.c. of cerebrospinal fluid were used instead of 1 c.c.

RESULTS

Group I: Normal.—Group 1 contains fluids whose cellular and other chemical contents were normal. The calcium values of 20 fluids of this group were found to vary from 4.1 to 5.9 mg. per cent, with an average value of 5.0 mg. per cent. Only 4 fluids had values greater than 5.5 mg. per cent, and 3 had values less than 4.5 mg. per cent. These values are in close agreement with the values for normal fluids given by Katzenellenbogen[1] and Merritt and Fremont-Smith,[5] and also for the values in epilepsy, idiocy, and cerebral birth trauma.

*From the Department of Biochemistry, Nelson Morris Institute for Medical Research, the Children's Hospital of the Cook County Hospital, and the Sarah Morris Hospital for Children of the Michael Reese Hospital.

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TABLE I

NORMAL CEREBROSPINAL FLUIDS

CASE	DIAGNOSIS	CELL COUNT	CALCIUM MG. PER CENT	PHOSPHORUS MG. PER CENT
B. B.	Epilepsy	1	5.0	1.3
R. P.	Epilepsy	3	4.5	1.4
J. B.	Epilepsy	4	4.7	
W. S.	Epilepsy	3	5.3	1.3
L. N.	Epilepsy	6	5.1	1.0
M. M.	Cerebral birth trauma	8	5.5	1.0
D. M.	Cerebral birth trauma	12	5.0	1.3
J. S.	Cerebral birth trauma	7	4.8	1.3
C. H.	Cerebral birth trauma	3	4.8	
A. F. (1)	Cerebral birth trauma	4	4.8	
A. F. (2)	Cerebral birth trauma		4.8	
N. J.	Cerebral agenesis	4	5.1	2.2
M. K. (1)	Cerebral agenesis	7	4.7	
M. K. (2)	Cerebral agenesis	0	5.7	1.5
S. S.	Idiocy	4	4.3	
D. R.	Idiocy	6	4.1	1.5
M. F.	Idiocy		4.4	1.2
B. A. F.	Idiocy		5.6	
B. B.	Idiocy	3	5.6	1.5
D. D.	Idiocy		5.9	1.1

TABLE II

TUBERCULOUS MENINGITIS

CASE	APPEARANCE OF FLUID	CELL COUNT	CALCIUM MG. PER CENT	PHOSPHORUS MG. PER CENT
C. W.	Clear	150	7.2	
L. W. (1)	Clear	220	5.5	
L. W. (2)	Pink (Prontosil)	160	6.7	1.8
L. S. (1)	Clear	450	6.0	2.0
L. S. (2)	Clear	450	6.7	2.0
L. S. (3)	Clear	590	7.0	3.4
R. C.	Opalescent	350	6.9	1.4
E. T.	Clear		6.7	1.8
G. R. (1)	Clear	175	5.9	1.8
G. R. (2)	Opalescent	180	6.5	2.1
G. R. (3)	Opalescent	850		2.1
G. R. (4)	Opalescent	700	6.4	2.2
C. F.	Clear	98	6.2	3.0
L. C.	Clear	190	5.2	
M. W.	Opalescent		5.8	
I. W. (1)	Clear	126	5.3	1.6
I. W. (2)	Clear		5.6	1.8
*R. M. P.	Clear	160	5.4	1.4
F. C.	Opalescent	290	6.7	2.2
B. D.	Clear	200	6.9	1.8

*Diagnosis not confirmed.

Thirteen fluids of this group were examined for their phosphorus content. In 12 fluids the phosphorus content lay between 1.0 and 1.5 mg. per cent; in one fluid a relatively high value of 2.2 mg. per cent was found. The average for the group was 1.4 mg. per cent. These values are also in close agreement with those given for normal fluids.[1, 5]

Group II: Tuberculous Meningitis.—The fluids from patients with tuberculous meningitis were generally found to have elevated calcium content. Nineteen fluids of this group were found to have calcium contents ranging from 5.2 to 7.2 mg. per cent, with an average value of 6.2 mg. per cent. Of these 19

fluids, 13 had values greater than 5.5 mg. per cent; only 3 had values less than 5.5 mg. per cent. It is of interest to note that when it was possible to obtain specimens of cerebrospinal fluid from a patient at different stages of the disease, the calcium values rose as the disease progressed. Thus, the calcium content of the fluid of patient L. W. rose from 5.5 to 6.7 mg. per cent; that of L. S., from 6.0 to 7.0; that of G. R., from 5.9 to 6.5; and that of I. W., from 5.3 to 5.6.

TABLE III

PURULENT MENINGITIS AND PYOCEPHALUS

CASE	DIAGNOSIS	APPEARANCE OF FLUID	CELL COUNT	CALCIUM MG. PER CENT	PHOSPHORUS MG. PER CENT
R. W.	Influenzal	Very turbid	5000 poly.	5.9	
R. H.	Influenzal	Very turbid		4.9	
W. G. (1)	Streptococcic (anhemolytic)	Very turbid	600 poly.	6.5	
W. G. (2)	Streptococcic (anhemolytic)	Very turbid, ventricular	4600 poly.		1.5
W. G. (3)	Streptococcic (anhemolytic)	Very turbid	5000 poly.		3.0
G. O. (1)	Streptococcic (hemolytic	Very turbid	8500 poly.	6.3	
G. O. (2)	and viridans)	Very turbid	720 poly.	6.4	
J. D. (1)	Streptococcic (hemolytic)	Very turbid	2290 poly.	5.6	
J. D. (2)	Streptococcic (hemolytic)	Opalescent	440 poly.	4.5	
J. B.	Staphylococcic	Turbid	6000 poly.		1.2
J. J.	Pneumococcic	Turbid	5000 poly.		1.2
D. R. (1)	Meningococcic	Turbid	3000 poly.	5.2	1.4
D. R. (2)	Meningococcic	Turbid	1200 poly.	4.9	1.4
D. R. (3)	Meningococcic	Opalescent	45 lymph.	4.0	2.1
K. S. (1)	Influenzal	Very turbid	2800 poly.		1.9
K. S. (2)	Influenzal	Opalescent	180 poly.	6.0	1.8
K. S. (3)	Influenzal	Opalescent	1650 poly.	6.0	
*R. K. (1)	B. pyocyaneus	Very turbid, ventricular, frank pus		6.6	7.0
R. K. (2)		Very turbid, ventricular, frank pus		8.7	
†R. K. (3)	B. pyocyaneus	Very turbid, ventricular, frank pus		10.2	7.4
R. K. (4)	B. pyocyaneus	Very turbid, ventricular, frank pus		12.3	9.2

*The cerebrospinal fluid protein values were very high in case (R.K.), being 2500, 2930, and 3810 mg. per cent in (1), (3), (4), respectively.

†The values of the serum calcium and phosphorus were normal, being 10.3 and 5.0 mg. per cent, respectively.

The phosphorus content of the fluids of this group, like that of the calcium, was generally elevated. The values found for 16 fluids ranged from 1.4 to 3.0 mg. per cent, with an average of 2.0 mg. per cent. In only 2 fluids was the phosphorus content less than 1.5 mg. per cent; in 7 it was 2.0 mg. per cent or higher. The phosphorus content of the cerebrospinal fluid also seemed to rise as the disease progressed. In one case, L. S., it rose from 2.0 to 3.4 mg. per cent; in the case of G. R., from 1.8 to 2.2 mg. per cent; and in the case I. W., from 1.6 to 1.8 mg. per cent.

Group III: Purulent Meningitis and Pyocephalus.—The fluids of this group were also found to be higher in calcium and phosphorus than the normal fluids. Of 16 fluids studied, 12 from patients with meningitis were found to range in

TABLE IV

HYDROCEPHALUS

CASE	APPEARANCE OF FLUID	CELL COUNT	CALCIUM MG. PER CENT	PHOSPHORUS MG. PER CENT
W. H. (1)	Opalescent, ventricular	310 Poly.	7.6	5.2
W. H. (2)	Opalescent, ventricular	350 Poly.	7.5	6.0
G. C.	Xanthochromic, ventricular		6.1	
W. W.	Xanthochromic, lumbar	40 Lymph.	6.9	2.6
B. S. (1)	Clear, ventricular		7.5	1.8
B. S. (2a)	Clear, lumbar	30 Lymph.	6.2	1.7
B. S. (2b)	Clear, ventricular	10 Lymph.	6.1	2.0
M. R. (1)	Clear, lumbar	20 Lymph.	5.2	2.1
M. R. (2)	Clear, lumbar	1 Lymph.	5.4	2.1
J. M.	Clear, ventricular	3 Lymph.	6.5	2.9
R. G. (1)	Clear, lumbar	2 Lymph.	4.9	
R. G. (2)	Clear, lumbar	2 Lymph.	4.4	1.2
N. L. W.	Clear, ventricular		6.3	1.2
B. G. B. (1)	Clear, ventricular		7.4	
B. G. B. (2)	Clear, ventricular		7.0	
B. B. L.	Xanthochromic, ventricular		7.6	

TABLE V

BRAIN TUMOR, BRAIN ABSCESS, BRAIN CYST

CASE	DIAGNOSIS	APPEARANCE OF FLUID	CELL COUNT	CALCIUM MG. PER CENT	PHOSPHORUS MG. PER CENT
J. M.	Cerebellar Glioma	Clear, ventricular	3 Lymph.	6.5	2.9
*E. P. (1)	Glioblastoma of left temporal lobe	Clear, lumbar	120 Lymph.	6.9	2.7
E. P. (2)	Glioblastoma of left temporal lobe	Clear, lumbar	150 Lymph.	6.7	2.2
E. P. (3)	Possible subarachnoid block	Yellow, lumbar, viscid	450 Red blood cells		1.7
E. P. (4)	Possible subarachnoid block	Yellow, lumbar, viscid		8.3	3.9
E. P. (5a)	Possible subarachnoid block	Yellow, lumbar, viscid	7 Lymph. Some red blood cells		4.5
†E. P. (5b)	Possible subarachnoid block	Clear, subarachnoid ?	7 Lymph.	5.6	1.6
‡E. P. (6a)	Possible subarachnoid block	Yellow, lumbar, viscid		8.3	4.0
†E. P. (6b)	Possible subarachnoid block	Subarachnoid ?	Some red blood cells	4.6	2.0
W. W.	Cerebellar medulloblastoma	Xanthochromic	40 Lymph.	6.9	2.6
C. K.	Cerebral abscess	Turbid	3000 Poly.		1.2
L. C. (1)	Cerebral abscess	Clear	170 Lymph.	5.6	1.1
L. C. (2)	Cerebral abscess	Clear	22 Lymph.		1.5
R. M. (1)	Cerebral subarachnoid cyst	Xanthochromic	0 Lymph.	5.0	
R. M. (2)	Cerebral subarachnoid cyst	Xanthochromic	110 Lymph.	6.6	
R. M. (3)	Cerebral subarachnoid cyst	Xanthochromic			1.9
R. M. (4)	Cerebral subarachnoid cyst	Xanthochromic		6.4	2.1

*The cerebrospinal fluid protein values for case (E.P.) were 207, 154, 3600, 2760, 3870, 28, 3930, and 27 for fluids 1, 2, 3, 4, 5a, 5b, 6a and 6b, respectively.

†From decompressed area above tumor.

‡The values of the blood serum calcium and phosphorus were 9.6 and 6.1 mg. per cent, respectively, i.e., normal.

value from 4.0 to 6.5 mg. per cent, with an average of 5.5 mg. per cent. In two instances in which patients recovered, J. D. and D. R., the calcium values decreased from 5.6 to 4.5 mg. per cent, and from 5.2 to 4.0 mg. per cent, respectively.

Phosphorus, in 9 fluids from patients with meningitis, ranged in value from 1.2 to 3.0 mg. per cent, with an average value of 1.7 mg. per cent.

Four fluids were obtained from patient R. I. with *B. pyocyaneus* pyocephalus. The calcium values found were 6.6, 8.7, 10.2, and 12.3 mg. per cent, increasing as the disease progressed. Phosphorus, determined in 3 fluids, had values of 7.0, 7.4, and 9.2 mg. per cent. Although on autopsy no meningeal exudate was found, pathologically the fluids closely resembled those of the purulent meningitides. For this reason they are included in group III.

Group IV: Hydrocephalus.—The calcium content of fluids from patients with hydrocephalus was found to show marked elevations. Sixteen fluids from 10 patients had values from 4.4 to 7.6 mg. per cent, with an average of 6.4 mg. per cent. Only 4 fluids from 2 patients with mild hydrocephalus fell within the range of normal values.

Phosphorus was determined in 11 fluids from 7 patients. Values ranged from 1.2 to 6.0 mg. per cent, with only two values within the normal range. The average value for the group was 2.6 mg. per cent.

Group V: Brain Tumor, Brain Abscess, Brain Cyst.—Most of the fluids in this group, like those of group IV, had elevated calcium and phosphorus contents. Twelve fluids were examined for calcium content and were found to have values ranging from 5.0 to 8.3 mg. per cent, with an average of 6.4 mg. per cent. In only two instances were the values less than 5.5 mg. per cent. A specimen obtained at a later date from patient R. M. showed an increase from 5.0 to 6.6 mg. per cent.

Phosphorus was determined in 15 fluids of this group; the values ranged from 1.1 to 4.5 mg. per cent, with an average of 2.4 mg. per cent. Eleven fluids of the 14 had values greater than 1.5 mg. per cent.

TABLE VI

MISCELLANEOUS

CASE	DIAGNOSIS	CELL COUNT	CALCIUM MG. PER CENT	PHOSPHORUS MG. PER CENT
C. W.	Cerebral arteriosclerosis	4	5.2	1.4
H. K.	Hemihypertrophy of the body	3	4.0	0.9
H. W.	Chorea	4	4.9	1.5
A. M. B.	Chorea	6	4.6	1.4
L. M.	Postencephalitis syndrome	12	5.8	1.2
T. W.	Postdiphtheritic tabes	8	4.8	
E. I.	Acute benign lymphocytic meningitis	290		1.2
L. T.	Preparalytic poliomyelitis	120		1.1
D. M.	Polioencephalitis ?	9	5.1	1.6
K. B.	Mumps meningoencephalitis	170	5.4	
L. W.	Postpneumonic encephalitis		4.6	1.8
A. W.	Miliary tuberculosis	200	5.1	0.8
C. T.	Typhoid	80		1.2
C. M.	Postmeasles encephalitis	1		1.0
B. S.	Postmeasles encephalitis	1	6.0	1.4

Group VI: Miscellaneous.—On the whole, the calcium and the phosphorus contents of the fluids of this group resembled those of normal fluids. The calcium values of 11 fluids ranged from 4.0 to 6.0 mg. per cent, with an average of 5.0 mg. per cent. In only two instances was the value greater than 5.5 mg. per cent. Phosphorus values, determined from 13 fluids, ranged from 0.8 to 1.8 mg. per cent, with an average of 1.3 mg. per cent.

TABLE VII

	CALCIUM MILLIGRAMS PER CENT			PHOSPHORUS MILLIGRAMS PER CENT		
	Minimum	*Maximum*	*Average*	*Minimum*	*Maximum*	*Average*
Group I						
Normal	4.1	5.9	5.0	1.0	2.2	1.4
Group II						
Tuberculous meningitis	5.2	7.2	6.2	1.4	3.0	2.0
Group III						
Purulent meningitis	4.0	6.5*	5.5*	1.2	3.0*	1.7*
		12.3†	7.1†		9.2†	3.3†
Group IV						
Hydrocephalus	4.4	7.6	6.4	1.2	6.0	2.6
Group V						
Brain tumor	5.0	8.3	6.4	1.1	4.5	2.4
Brain abscess						
Brain cyst						
Group VI						
Miscellaneous	4.0	6.0	5.0	0.8	1.8	1.3

*Excluding the values for fluids from patient (R.K.).
†Including the values for fluids from patient (R.K.).

DISCUSSION

The results of our work indicate that the cerebrospinal fluid calcium and phosphorus are generally elevated in tuberculous and purulent meningitis, hydrocephalus, brain tumor, and brain cyst. Some of the possible causes of these increases will be discussed briefly.

Probably the chief cause of increased cerebrospinal fluid calcium and phosphorus in meningitis is the increased meningeal permeability caused by the infection. It is possible to understand on this basis the increase in the calcium and phosphorus values with the progress of the disease. Thus an increase in the length of time, during which the meningeal permeability has been elevated, tends to increase the amounts of calcium and phosphorus reaching the subarachnoid space from the blood. The opposite may be the case if the progress of the disease is arrested and the patient recovers. However, it is difficult to understand why, in some cases of purulent meningitis (Table III, R. H., J. D., D. R.), the calcium values are not elevated, even though on recovery (J. D., D. R.) the values did decrease. Similarly, the phosphorus values were sometimes normal in severe cases of purulent meningitis. Also it is interesting to note that the calcium and phosphorus values were more consistently high in tuberculous meningitis than in purulent meningitis. This may be due to the fact that the disease ran a longer course in the case of tuberculous meningitis than in purulent meningitis. It is possible that other factors besides increased meningeal permeability are involved. It has been reported[6, 7] that in cases of tuberculous meningitis, there is often an extension of the inflammatory process into the cortex, resulting in foci of encephalitis. Whether the resulting brain

tissue destruction may be related to the consistently elevated calcium and phosphorus values of tuberculous meningitis is as yet an open question.

The fluids from patients with hydrocephalus and brain tumor fell into two groups; those which were xanthochromic and had high protein content, and those which were clear and relatively low in protein content. The fluids of the former group all had high calcium content; some of the fluids of the latter group were also high in calcium content; others were normal. Xanthochromic fluids were obtained from patients who were found on autopsy to have had lesions which invaded the meninges or ventricles. Walter[8] has reported that in such cases there is increased meningeal permeability. In the fluids of this type, the high calcium and phosphorus content may also be due in part to brain tissue destruction.

The mechanism by which the cerebrospinal fluid calcium and phosphorus may be increased during the course of brain tissue destruction is not clear. Recent work[9] indicates that the calcium content of fresh normal brain tissue is about 4 mg. per 100 gm. The complete degeneration of 100 gm. of brain tissue could have no significant effect on the calcium content of the cerebrospinal fluid, if those figures are correct. It follows that the brain tissue cannot be the source of the increase in cerebrospinal fluid calcium. It is known, however, that degeneration of tissue is often followed by calcification. Wells[10] stated it as a rule that, except for the metastatic calcification of Virchow, degeneration always precedes pathologic calcification. The calcium salts involved come from the blood vessels of the degenerating tissue. One example of this type of pathologic calcification is that found in ganglion cells of the brain which have become degenerated or necrotic. Further, Geyelin and Penfield[11] have reported the occurrence of a group of cases of familial cerebral calcification with epilepsy. They considered that the pathologic process underlying the group of cases was a slowly progressive closure of the end arteries in the cerebrum which produced an area of local necrosis which then become calcified. Whether calcification actually does occur in an area of degeneration or not, it is possible that there is an accumulation of calcium salts in that area. Some of those salts may be carried to the subarachnoid space via the perivascular spaces. In cases where there is no evidence of increased meningeal permeability, the increased calcium content of the cerebrospinal fluid may be an index of calcium salt accumulation in an area of brain tissue degeneration. This hypothesis is in agreement with the fact that in cases of severe hydrocephalus with much cortical destruction high calcium values were found; in cases where encephalography showed relatively slight degeneration, calcium values were normal.

The phosphorus content of fresh normal white matter is, according to Alexander and Myerson,[9] about 530 mg. per 100 gm.; that of gray matter is about 180 mg. per 100 gm. Since cerebral pressure atrophy involves chiefly the white matter,[12] the destruction of large amounts of brain tissue could contribute significantly to the phosphorus content of the cerebrospinal fluid. It is probable that some phosphorus accompanies the calcium which comes from the blood vessels. There are, therefore, two possible sources of phosphorus, so that together with increased calcium values, increased phosphorus content of the cerebrospinal fluid may be an index of brain tissue destruction.

The clinical usefulness of our results cannot be definitely evaluated as yet. In tuberculous meningitis the elevation of calcium and phosphorus, together with other chemical findings, may be of diagnostic help. In purulent meningitis the calcium and phosphorus values are not sufficiently consistent to be of much aid clinically. In hydrocephalus and brain tumor high calcium and phosphorus values may indicate brain tissue destruction and may be of prognostic value in those conditions.

CONCLUSIONS

1. The calcium content of normal cerebrospinal fluid was found to vary from 4.5 to 5.5 mg. per cent, with an average of 5.0 mg. per cent. The phosphorus content varies from 1.0 to 1.5 mg. per cent.

2. Calcium and phosphorus were found to be consistently elevated in tuberculous meningitis, the values increasing with the progress of the disease.

3. Calcium and phosphorus values were occasionally elevated in purulent meningitis, but not so consistently as in tuberculous meningitis.

4. Calcium and phosphorus were generally elevated in hydrocephalus and in brain lesions.

5. The causes of elevated calcium and phosphorus values are probably increased meningeal permeability and brain tissue destruction. The latter may be of particular interest in hydrocephalus and brain tumor.

6. The elevation of cerebrospinal fluid calcium and phosphorus may be of corroborative value in the diagnosis of tuberculous meningitis and may yield some information about the destruction of brain tissue in hydrocephalus and brain tumor.

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A PRELIMINARY REPORT OF THE BLOOD PICTURE IN BRUCELLOSIS*

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THERE are scattered reports in the literature to the effect that the blood picture in undulant fever is characterized by a leucopenia with a relative lymphocytosis,[1, 2] a slight to moderate secondary anemia, and a color index of less than one.[1] There has been no extensive study of the blood picture in many cases of undulant fever from which one of the species of *Brucella* was isolated.

An opportunity presented itself this year to study the blood picture on a number of acute undulant fever cases from which *Brucella melitensis* was recovered. The patients were located on the island of Malta. All patients were showing symptoms of the disease at the time of examination. The blood examinations were made from two to four weeks after the onset of the disease.

The data from 10 cases in Table I are representative of the findings from 32 cases studied.

Size of Red Blood Cells.—We have been unable to find any references pertaining to the size of the red blood cells in undulant fever. A study of their size in these cases revealed that there is a marked variation in the blood of undulant fever patients from the normal. In 35 per cent of the patients studied it was found that an increased number of the cells were smaller than normal, and in 19 per cent there was a tendency of the cells to be larger than normal. The average red blood cell measurement formula was of a 39:37:24 ratio instead of the 33:34:33 ratio[8] which we are using as the normal distribution. The red blood cells of some patients varied in size from 3 to 10 microns, although the normal variation is from 6 to 10 microns. This variation in size is probably effected by an accompanying splenic and liver pathology.

White Blood Cells.—The white blood cell count on the average is lower than normal with a relative and absolute monocytosis.[3] Qualitatively we find an increase of the nonfilamented neutrophiles. For several hours after injections of brucellin (the curative agent) there is an elevation of the leucocyte count due to the increase of the nonfilamented and filamented neutrophiles. The blood picture does not return to normal until recovery has taken place.

One interesting point that has not been noted before is that many of the mature small lymphocytes are much larger than normal which is similar to the infectious mononucleosis type of lymphocyte. These have been termed by Calder[4] "pathologic lymphocytes," and others have named them "large mature lymphocytes." These cells have all the appearance of small lymphocytes, the ratio of the nucleus to cytoplasm being similar to that of the normal small lymphocyte. They are about 12 to 14 microns in diameter. The normal small

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TABLE I

CASE NO.	W. B. C.	DIFFERENTIAL COUNT PER CENT							LVD*	BGN*	RED BLOOD CELL MEASUREMENT		
		EOSINOPHILES	BASOPHILES	NONFILAMENTED NEUTROPHILES	FILAMENTED NEUTROPHILES	LARGE LYMPHOCYTES	SMALL LYMPHOCYTES	MONOCYTES			7.5 μ <	7.5 μ	7.5 μ >
3 (2)	5,000	1	2	12	16	49	10	10	2	4+	21	38	41
6 (1)	4,600	0	0	21	10	48	4	17	3	3+	51	34	15
7 (1)	5,200	0	0	29	6	47	7	11	2	2+	45	35	19
9 (2)	4,600	0	0	27	12	49	6	6	2	3+	38	40	22
12 (1)	5,025	0	0	30	27	31	4	8	1	2+	75	26	5
13 (1)	4,100	0	0	30	23	36	4	7	2	3+	27	52	21
15 (1)	2,800	0	0	26	23	38	6	6	2	4+	34	40	26
17 (1)	3,300	0	0	31	20	40	2	7	1	3+	34	39	27
17 (2)	3,500	0	0	15	30	40	4	11	1	2+	33	34	33
18 (2)	4,900	0	0	31	21	36	3	9	3	1+	32	33	35
Average	3,850	0.1	0.2	25.2	18.8	41.4	5.0	9.2	1.9		39.0	37.0	24.0
Normal	8,000	1	0.5	6	60	8	20	4.5	0	0	33	34	33

*LVD = Liver damage cell.
BGN = Basophilic granulation of the neutrophiles.

lymphocytes usually measure from 8 to 10 microns. As many as 30 to 80 per cent of the lymphocytes may appear as this type of large cell in about 40 per cent of the brucellosis patients.

Sabin[5] by supervital staining has found, in cases of brucellosis, an increase in the type of monocyte which is similar morphologically to the type of monocytes associated with various forms of hepatic involvement.[6] These appear to be similar to the atypical monocytes found in catarrhal jaundice.[5] In disease associated with liver pathology, Isaacs[7] has described "a cell averaging 15 by 13 microns, with an oval nucleus, rather dense chromatin (lymphoid in character), foamy blue-staining cytoplasm (monocytoid), but with absence of the minute, red staining granules of the monocyte. There is no perinuclear clear zone, as in the lymphocyte. Occasional inclusion granules are found in the cytoplasm. The margin is wavy." The "liver damage cell" of Isaacs was present consistently in all of the cases of brucellosis examined.

Another interesting point that has been noted in these Maltese patients is that in *Brucella melitensis* infection there is a marked basophilia of the granules of the neutrophiles. This phenomenon may be associated with the temperature elevation that the patients experienced. We believe that this is characteristic of the *Brucella melitensis* infection, since we have not encountered basophilia of the granules in *Brucella suis* and *Brucella abortus* infections. These granules are similar in size to the *Brucella* bacteria and stain similarly. It is suggested that a control plain smear always be made without the bacteria when a phagocytosis test is performed. A toluidine blue staining technique has been perfected by the junior author for the staining of phagocytosis smears from *Brucella melitensis* infected patients.

SUMMARY AND CONCLUSION

We have found in the study of *Brucella melitensis* infected individuals that the blood picture reveals a leucopenia with a relative lymphocytosis and monocytosis.

The red blood cells tend to be slightly smaller than normal; however, some patients gave evidence of macrocytosis.

The presence of "pathologic lymphocytes" in 40 per cent of the brucellosis cases is significant.

"Liver damage cells" were found consistently in these patients.

Finally the basophilia of the granules of the neutrophiles seems to differentiate the *Brucella melitensis* infection from that of the *suis* and the *abortus* infection.

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RELATIONSHIP BETWEEN AGGLUTINABILITY AND CERTAIN IN VITRO TESTS OF STAPHYLOCOCCI, STREPTOCOCCI, AND COLON BACILLI ISOLATED FROM PERSONS SUSPECTED OF HAVING CHRONIC INFECTION*

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AGGLUTINATION reactions have been used in attempts to appraise the significance of bacteria isolated from suspected foci of infection. In efforts to determine the reliability of these reactions it was reasoned that pathogenicity of the organisms should play a part in their relationship to the disease process. However, it would have been impractical to make animal inoculation tests on the twelve thousand cultures of staphylococci, streptococci, and colon bacilli used in these experiments. Therefore, the following tests were used because they had previously been shown to have given results parallel with certain pathogenic properties of the cultures. Pigment, hemolysis, and coagulase tests[1] were used for staphylococci; resistance to the bactericidal action of fresh, diluted, defibrinated guinea pig blood[2-4] was used for streptococci; and the electrophoretic migration velocity[5, 6] was used for the colon group. These will be referred to as in vitro tests.

For the agglutination reactions, pure cultures of streptococci were grown in brain heart infusion overnight, tested for purity, washed once with 1.0 per cent phenol in 0.85 per cent salt solution, and resuspended in it. The final concentrations were about 10 billions per c.c. Other bacteria were grown on solid media and suspended in the phenol saline without washing. Unless the suspensions were decanted before use, the control tubes often contained a deposit of bacteria and extraneous matter which was difficult to distinguish from specifically agglutinated particles. The methods described by Spicer[7, 8] and Mueller and Klise[9] were found useful for preparing suspensions of autoagglutinative cultures.

A series of tubes containing 1.0 c.c. of progressive dilutions of the patient's serum, ranging from 1:40 to 1:5,120, together with a saline control, were placed in a rack. To each tube was added 1.0 c.c. of the bacterial suspension. After being shaken for two minutes, the tubes were placed in a water bath at 50° to 55° C. for one hour. The tubes were inspected periodically, and the results read whenever the control tube began to show excessive sedimentation. Otherwise, the rack was left on the laboratory table until the following morning, and the results read at that time.

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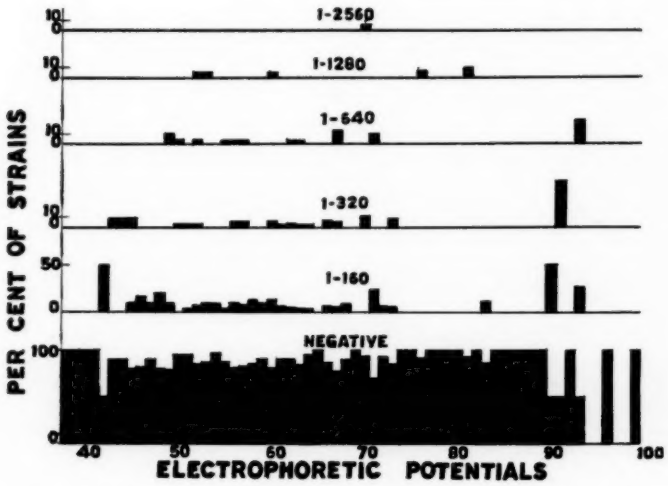

Fig. 1.—Comparison of electrophoretic migration velocity and agglutinability of 893 *B. coli-aerogenes* cultures.

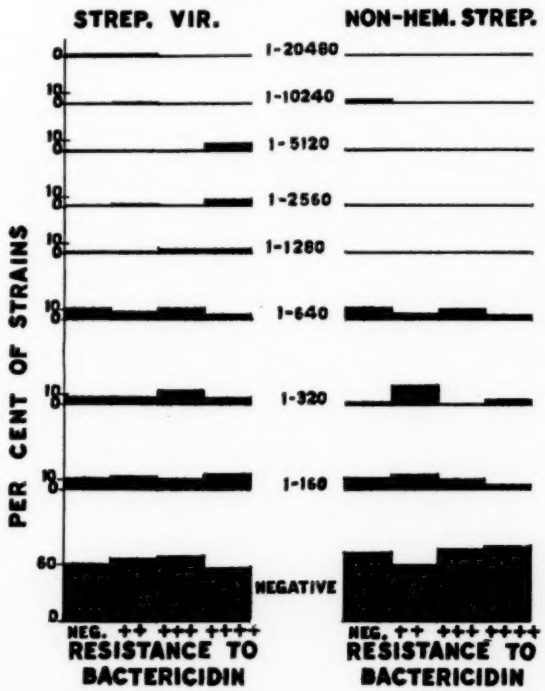

Fig. 2.—Comparison of resistance to the bactericidal action of fresh, diluted, defibrinated guinea pig blood and agglutinability of 627 cultures of *Streptococcus viridans* and 107 cultures of gamma type streptococci.

Some of the tests gave an increased titer when re-examined. This was found to have been caused by jarring the tubes when they were dropped back into the racks. In subsequent tests the tubes were lifted about 1 cm. and dropped back into the racks, and this was repeated a short while later. The results were read soon afterward. Because many of the sera agglutinated most of the organisms in dilutions up to 1:80, only reactions of 1:160 and over were considered positive.

Certain sera agglutinated a wide variety of bacteria, some of them in high dilution. Other sera gave only weak reactions. This suggested that agglutination was as much a function of the serum as of the bacteria.

The proportion of agglutinable cultures in different groups was as follows: *Staphylococcus aureus*, 47 per cent; hemolytic streptococci, 42 per cent; *Streptococcus viridans*, 36 per cent; *Staphylococcus albus*, 32 per cent; gamma type

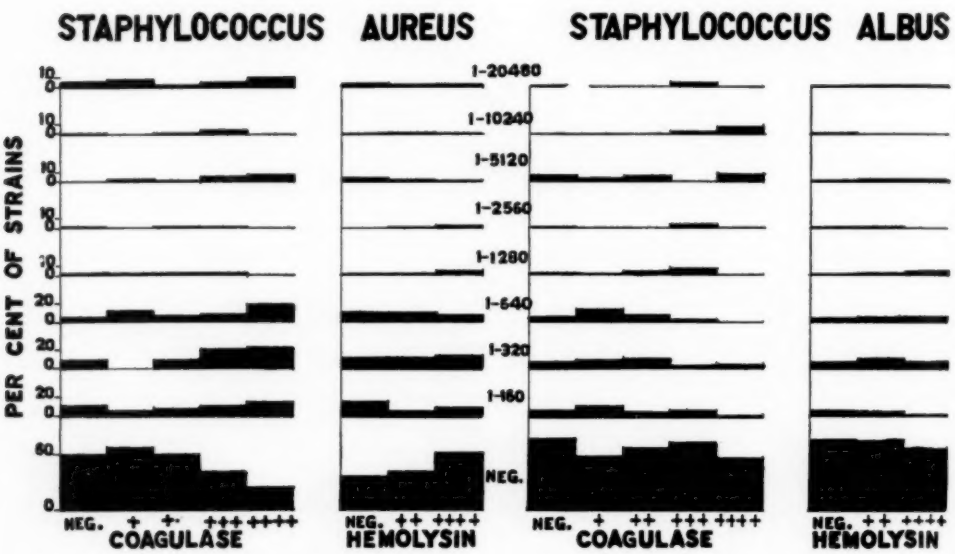

Fig. 3.—Comparison of hemolytic and coagulating properties with the agglutination titer of 368 cultures of *Staphylococcus aureus* and 885 cultures of *Staphylococcus albus*.

streptococci, 28 per cent; gamma type enterococci, 25 per cent; colon bacilli, 14 per cent; and alpha type enterococci, 10 per cent. In a series of 110 normal adults, Steinberg and Wiltsie[10] obtained with colon bacilli a titer of 1:100 in 3, 1:300 in 4, and 1:1,000 in 1 person.

TABLE I

RELATIONSHIP BETWEEN AGGLUTINABILITY AND IN VITRO TESTS OF BACTERIA ISOLATED FROM PERSONS SUSPECTED OF HAVING FOCAL INFECTION

TYPE OF ORGANISM	CULTURES EXAMINED	IN VITRO POSITIVE		IN VITRO NEGATIVE		PER CENT AGREEMENT (COLUMNS 1 + 4)
		AGGL. + (1)	AGGL. 0 (2)	AGGL. + (3)	AGGL. 0 (4)	
Staphylococcus albus	2975	7.2	11.3	16.3	65.3	72.5
Staphylococcus aureus	1122	52.3	40.6	3.5	3.7	56.0
Hemolytic streptococci	309	26.4	32.3	21.3	19.7	46.1
Nonhemolytic streptococci	861	13.8	29.8	16.7	39.7	53.5
Streptococcus viridans	4980	19.5	39.9	18.2	22.8	42.3
Colon bacilli	2148	1.9	20.4	12.2	65.5	67.4

Reactions considered positive:
Staphylococci—any strain which coagulated human and rabbit plasma.
Streptococci —cultures giving ++++ and +++ resistance to fresh, diluted defibrinated guinea pig blood (see references 2 and 3).
Colon bacilli —electrophoretic migration velocities of less than 52 microns/100 volts/sec./3.45 cm. (see references 4 and 5).
Agglutination—titers of 1:160 and over.

There did not seem to be a relationship between the agglutination titer and the strength of the in vitro reaction (Figs. 1, 2, and 3). Therefore, it will simplify comparison to consider only qualitative relationships between the in vitro tests and agglutinability (titers of 1:160 and over). These showed agreement in 72.5 per cent of *Staphylococcus albus* and in 67.4 per cent of colon bacilli, but other groups showed agreement in only about one-half of the cultures (Table I).

Agglutinable cultures did not give stronger in vitro reactions than inagglutinable cultures. Certain cultures which gave strongly positive in vitro reactions were inagglutinable. Rawls and Chapman[2] found that, when a streptococcus which was resistant to fresh guinea pig blood was not agglutinated by the patient's serum, it had greater power to produce arthritis in rabbits than did similar but agglutinable strains.

CONCLUSIONS

In vitro tests which had given results parallel with certain pathogenic properties were applied to cultures of staphylococci, streptococci, and colon bacilli isolated from persons suspected of having chronic infection. The cultures were also tested for agglutinability by the serum of the person from whom they had been isolated.

There was no close relationship between the in vitro reaction and the agglutination titer.

There was agreement in 67.4 per cent of colon bacilli and in 72.5 per cent of *Staphylococcus albus*, but there was agreement in only about one-half of the cultures of streptococci and *Staphylococcus aureus*.

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SODIUM DEHYDROCHOLATE SOLUTION AS A SOLVENT FOR NEOARSPHENAMINE IN THE TREATMENT OF SYPHILIS*

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IN THE course of the treatment of syphilis with the trivalent arsenicals one occasionally finds patients who have a persistent gastrointestinal intolerance for these drugs. Although the number of such patients is small, they nevertheless present an important therapeutic problem because of the necessity of continuing arsenical treatment in some of them.

In general, there are two types of gastrointestinal reactions: immediate and delayed. The former type, occurring during or shortly after the administration of these drugs, may be due to a number of factors, such as gagging produced by the characteristic odor of these drugs, "speed" shock, colloidoclastic shock, "tubing" reaction, nitrotoid reaction, or the patient having eaten just prior to receiving the drug. For the most part, simple well-known measures will alleviate these reactions.

The delayed type of gastrointestinal reaction, which occurs two to five or more hours after treatment, is not so easily prevented. Changing from one drug to another, although frequently successful, does not always solve the problem. These delayed reactions are thought to be due to the arsenicals temporarily interfering with normal liver function.[1, 3]

With this in mind, it has been suggested that using as a vehicle for the arsenical a preparation which increases bile flow, such as sodium dehydrocholate, would help prevent reactions due to biliary dysfunction. Appel[2, 3] demonstrated the value of this drug in the treatment of arsenical hepatitis and as a solvent for neoarsphenamine in patients who did not tolerate neoarsphenamine dissolved in distilled water. Savulescu[4] and Costinescu[5] have shown that neoarsphenamine dissolved in a 5 per cent sodium dehydrocholate solution is therapeutically active. It is thought that this combination of drugs results in a mixture rather than a new chemical compound.

In an effort to further evaluate sodium dehydrocholate, we selected a group of male patients who had been unable to tolerate one or all of the trivalent arsenicals because of gastrointestinal reactions. Only those patients who received at least five doses of neoarsphenamine dissolved in the sodium dehydrocholate solution have been selected. Because the group was small, we tried the use of the new vehicle in intolerant patients before changing to another drug. In all patients the technique of administration was identical with or without the sodium dehydrocholate. Care was taken to avoid undue aeration and to give all

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injections slowly. Patients selected were treated with gradually increasing doses of neoarsphenamine dissolved in a 5 per cent solution of sodium dehydrocholate (decholin sodium*). Five cubic centimeters of the 5 per cent solution were used for every 0.15 gm. of neoarsphenamine.

In a group of five patients in whom nausea and vomiting occurred immediately after treatment, sodium dehydrocholate was ineffective in preventing these reactions. If the mixture did not succeed on the first trial, it was not tried again and an attempt was made to change to another arsenical.

TABLE I

INCIDENCE OF TOLERANCE OF NEOARSPHENAMINE DISSOLVED IN SODIUM DEHYDROCHOLATE SOLUTION

PATIENT'S NUMBER	NUMBER INJECTIONS WITH SODIUM DEHYDROCHOLATE	NUMBER WELL TOLERATED	NUMBER NOT TOLERATED	SUBSEQUENTLY TOLERATED MAPHARSEN
177533	10	10	–	Yes
173555	15	12	3	
170757	11	9	2	Yes
177494	13	13	–	
179108	8	8	–	
178684	8	7	1	Yes
16630	11	10	1	
175189	8	8	–	Yes
163874	10	10	–	
168783	5	5	–	Yes
166534	5	5	–	Yes
164745	6	6	–	
Total	110	103	7	6

In a group of twelve patients with the delayed type of gastrointestinal reaction to neoarsphenamine, the results were much better. Out of a total of 110 injections given, 103 were well tolerated, i.e., there was no nausea or vomiting. However, six of the twelve patients were later able to tolerate mapharsen without gastrointestinal reactions. Table I illustrates the results in those patients in whom delayed nausea and vomiting occurred.

DISCUSSION

Five per cent sodium dehydrocholate solution as a solvent for neoarsphenamine was without value in those patients in whom nausea or vomiting occurred shortly after treatment; but in those patients in whom these reactions were delayed, it was capable of preventing reactions in 103 out of 110 trials. In two patients the drug was not permanently effective. After these patients had been satisfactorily carried on for eight doses with the mixture, they again became intolerant. No attempt was made to resume the use of sodium dehydrocholate after a rest in these two individuals. (Occasionally a patient would become nauseated some time during a course of neoarsphenamine dissolved in sodium dehydrocholate solution, but usually the next injection of the same mixture would be well tolerated.) In six patients the drug was discontinued after a reasonable trial—at least five doses—and mapharsen was used without reactions.

The added expense of using sodium dehydrocholate must be considered. However, in those cases where arsenicals are essential and where they can be used

*Supplied through the courtesy of Riedel-de Haen, Inc.

only when dissolved in a solution of this or a similar drug, the difference in the cost can be compensated for by the greater therapeutic value of the arsenicals as compared with prolonged use of heavy metals.

SUMMARY

In those patients who develop nausea and vomiting two to five hours after receiving an injection of neoarsphenamine, the reaction is thought to be due to the hepatotoxic character of the arsenicals.

Twelve such patients were given 110 doses of neoarsphenamine dissolved in a 5 per cent solution of sodium dehydrocholate (decholin sodium). Five cubic centimeters of sodium dehydrocholate solution were used for every 0.15 gm. of neoarsphenamine. One hundred and three of the 110 injections were well tolerated, i.e., no nausea or vomiting occurred. Six of the twelve patients were later able to tolerate a different drug (mapharsen). In two patients neoarsphenamine dissolved in sodium dehydrocholate solution was well tolerated at the beginning of the course but later there was the same reaction that occurred without the use of the sodium dehydrocholate.

Sodium dehydrocholate was valueless in preventing reactions in those patients in whom nausea and vomiting occurred immediately after treatment with neoarsphenamine.

Five per cent sodium dehydrocholate solution used as a solvent for neoarsphenamine widens the range of use of this drug in that it permits further use of this arsenical in those patients who develop the delayed type of gastrointestinal reaction.

The author is greatly indebted to his chief, Dr. J. E. Kemp, for valuable suggestions and permission to do this work.

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LABORATORY METHODS

INTRADERMAL TEST AS AN AID IN THE DIAGNOSIS OF ENTEROBIASIS*

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DESPITE the common occurrence of enterobiasis its clinical diagnosis is frequently open to errors chiefly due to difficulties in differentiating pruritis ani accompanying this infection from that attributable to other disturbances. The detection of ova of *Enterobius vermicularis* in the stool is likewise confronted with failure. Thus, according to Craig and Faust,[1] the ova of the parasite were found only in less than 5 per cent of infected individuals. In order to facilitate the diagnosis, therefore, additional methods have been suggested; namely, by the finding of the ova in perianal swabs such as recently introduced by Hall[2] or from under the fingernails, or by the recovery of adult worms by means of enemas. Though these methods are apparently superior to fecal examination, lack of proper cooperation on the part of patients may become a source of error in the diagnosis.

Enterobius vermicularis is generally considered as a strictly lumen parasite of the intestinal tract. The clinical pictures due to the presence of the parasites are at times not clear-cut. However, catarrhal inflammation of the immediate tissues caused by undue attachment of the worms may induce the absorption of toxins secreted by the worms. This may lead to nervous and systemic disturbances often observed in the patients. Schröpl[3] was of the opinion that the parasite secretes a toxin and that the frequency of the parasite and its connection with obscure common complaints should always be investigated. For this reason, the present study was undertaken to determine the relative value of the intradermal test as an aid of establishing the diagnosis of enterobiasis.

METHODS AND OBSERVATIONS

Grübel[4] was the first to introduce the intradermal test in the diagnosis of enterobiasis. The antigen prepared by him consisted of a saline emulsion of *Enterobius vermicularis*. He demonstrated that an immediate reaction developed in infected individuals within five minutes, with a progressive itching accompanied by a rash (Quaddelbildung). He also found that the extract was reactive after heating to boiling point. Schröpl later, by using the alcoholic extract of the worms, reported that a delayed reaction was obtained in 13 out of 15 infected

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individuals, while individuals with both negative history and stool gave no reactions. Recently, Wright and Bozicevich[5] compared the value of dermal and intradermal tests by the use of physiologic or buffer saline extracts of the worms, and claimed the higher specificity for the dermal test.

Preparation of the Antigen.—A large number of adult worms were recovered from the stool of a patient following purgation with a dose of magnesium citrate. The worms were carefully washed one by one by first dipping into several changes of sterile normal saline solution, and finally washing them down rather forcibly with the solution. The worms were then placed in a sterile Petri dish and left standing in an incubator at 37° C. until dried. These were further placed either in a vacuum over sulfuric acid or treated by freezing with lyophile apparatus until thoroughly dried, after which they were pulverized. The latter was extracted with absolute alcohol at room temperature for two days. The residue after evaporation was completely dried and then extracted for forty-eight to seventy-two hours with Coca solution (sodium chloride 0.7 per cent, sodium bicarbonate 0.05 per cent, and phenol 0.33 per cent in distilled water). After centrifuging, the supernatant fluid was drawn off, filtered through a Seitz filter, and stored in ampoules or vials in the refrigerator.

SKIN REACTION IN EXPERIMENTAL ANIMALS

With the use of the antigen thus prepared, three rabbits (one naturally and two experimentally infected with *Passalurus ambiguus*) were tested. The tests were made on the abdomen of the animal with Coca solution as a control. The results showed that the naturally infected animal gave uniformly positive reactions of more or less delayed type with 0.1 c.c. of 1:100, 1:500, and 1:1000 dilutions of the antigen, but not with 1:2000 dilution. The reactions developed one hour after the injection and persisted usually for twenty-four hours, at which time, however, erythema completely disappeared, leaving only a slightly edematous area. Control animals which were tested invariably gave negative reactions. The two experimentally infected animals showed the initial skin response on the thirty-first and thirty-fourth days, respectively, following the oral administration of washed ova of the parasites. One of the animals was later treated twice with hexylresorcinol. When no ova were recovered from the fecal pellets upon repeated examinations, the animal was removed to a new cage in order to prevent a possible re-infection. This animal was subsequently given by stomach tube 2000 trichinella larvae isolated from infected muscle by artificial digestion. The skin test with the enterobius antigen became negative three months later, while the test with trichinella antigen (1:1000) prepared by McCoy, Miller, and Friedlander[6] gave a definitely positive reaction. This result seemed to indicate that no group reaction occurred in the animal with trichinella infection by the use of the enterobius antigen. All the rabbits also harbored oocysts of *Eimeria stiedae* which indicated that the reaction was apparently specific for *Passalurus ambiguus*. In order to further determine the specificity of the antigen, 8 guinea pigs were experimentally infected with trichinella larvae. After an elapse of three weeks, the animals were tested with the enterobius antigen and found to be negative in all instances. In the fecal pellets of these animals were intestinal protozoa including balantidia.

SKIN REACTIONS IN MAN

Patients with the complaints of pruritis ani were tested with 0.05 c.c. of 1:500 dilution of the enterobius antigen on the flexor surface of the forearm. A control was run with Coca solution to eliminate certain type of pseudo-positives. If the reaction was positive, differential blood counts were made, emphasis being placed on the percentage of eosinophilic leucocytes. They were requested to submit to us a complete bowel movement following purgation with magnesium citrate or a copious enema. Careful search for adult worms was then instituted. This was done by floating out in clear water the material caught in the sieve (40 meshes per inch) and searching for the worms in a thin layer in the flat bottom of Petri dish over a dark background.

The positive skin reactions obtained are of immediate type and usually begin to develop in from five to ten minutes. They are characterized by the formation of a wheal of varying size with irregular pseudopodial margin surrounded by a zone of erythema. The reactions are usually read after twenty minutes. No test is considered positive in which there is not a definite wheal at least 7 mm. in diameter and a zone of erythema not less than 20 mm. in diameter. Definite positive reactions are quite clear with a wheal 10 to 20 mm. in diameter surrounded by an erythema up to 50 mm., with or without itching. Tentatively an erythema of 25 to 30 mm. in diameter is designated as + reaction; 35 mm., ++; 40 mm., +++; and 50 mm. or over, ++++ reactions. The reaction, as a rule, disappears within an hour or two. A slight brown discoloration of skin at the site of injection has often been observed by one of us (T. C. B.). Whether it represents dermatropic necrotizing effect of the toxins liberated by the worms is difficult to determine at this time.

The results of the study showed that 10 control persons who have had neither clinical symptoms nor the presence of the parasite in the stools showed negative skin reactions. Five persons with pruritis ani gave positive reactions, but no parasites were found upon repeated examinations. This may be explained by the fact that some individuals are hypersensitive to any proteins injected into the skin, or probably the reaction may be due to the comparative low dilution of the antigen used. Augustine and Theiler,[7] in their work on trichinosis, cited instances in which the number of positive reactions decreased when high dilutions of the antigen were used.

Nineteen cases with complaints of pruritis ani and the presence of the adult worms in the postcathartic stools showed positive reactions within twenty minutes following the injection of the antigen. In none of these cases were the number of eosinophiles over 2 per cent, and in one instance no eosinophiles were present. This seemed to indicate that no apparent positive correlation existed between the number of eosinophiles and enterobius infection. Paulian[8] claimed the presence of eosinophilotactic substances in the blood of infected individuals. These substances were found by him to be alcohol and water soluble. One of the cases under observation gave a negative reaction with a positive stool at the first visit but developed a strongly positive reaction two weeks later, indicating that the skin response to the enterobius antigen did not develop in the early stage of the infection.

Our observations lead us to believe that those who have had the infection and have been cured are apt to show a slightly positive reaction over a long period of time. It is for this reason that at least a two-plus reaction only should be considered diagnostic. The reaction, as a rule, begins to fade quite promptly after recovery, maintaining, however, a very low grade of response for some time to come.

COMMENT

The mechanism of the intradermal test in this and other helminthic infections is little understood. The test depends either upon hypersensitivity to certain materials contained in the antigen or upon injury of normal tissue by a definite toxin in the absence of specific antibody. It is highly probable that in individuals harboring pathogenic parasites, elaboration of antibodies has sensitized the skin. As a result, the introduction of a minimal amount of antigen derived from the homologous, or a closely related species, gives rise to an immediate local response. That the group specificity apparently exists in the case of *Enterobius vermicularis* was well demonstrated in the instances of rabbits infected with *Passalurus ambiguus.*

Judging from the results obtained in this study, we may say that the intradermal test is probably of value in diagnosing cases of enterobiasis in which pruritis ani is a predominant symptom (19 out of 24). Five cases with pruritis ani which gave positive skin reactions and negative stools, may be referable to disturbances other than of helminthic origin, such as neurologic or allergic conditions, or else fungoidal etiology may be sought for in this connection. In control persons, the test was specific in that none responded. Though the number of normal individuals tested in this series was too few to justify any conclusion, the test appeared to be useful in eliminating the possibility of enterobiasis.

From the practical point of view, the intradermal test in enterobiasis cannot be very well considered a certain diagnostic method. It should always be checked with parasitologic proof obtained by the methods previously described. The diagnosis, in our opinion, may be best made by searching for the adult worms in the posteathartic stool, or one following a copious enema.

SUMMARY

1. Experimental study made with enterobius antigen showed that 3 rabbits infected with *Passalurus ambiguus* gave definitely positive reactions with 1:100, 1:500, and 1:1000 dilutions, and not with 1:2000 dilution of the antigen. This illustrates an instance of group-specific reactions. One of the animals which was later treated and apparently recovered from the infection gave negative reaction along with the control animals. Furthermore, one rabbit and 8 guinea pigs infected with trichinella larvae did not respond to the enterobius antigen, indicating the specificity of the reaction.

2. Thirty-four individuals were tested with the enterobius antigen. Ten control persons gave negative reactions. Of 24 persons with complaints of pruritis ani, 19 developed positive reactions and the worms were found in the stools, while five gave positive reactions and negative stools. In one case of enterobiasis no reaction was elicited until two weeks after the recovery of the adult worms in posteathartic stools. This seemed to suggest that the tissue response to the enterobius antigen was lacking in the early stage of the infection.

3. Negative tests were apparently more valuable in indicating evidence of freedom from enterobiasis than were the positive ones in establishing it. However, the test should always be checked with parasitologic examination.

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A NEW INSTRUMENT FOR AUTOMATICALLY RECORDING THE ERYTHROCYTE SEDIMENTATION RATE AND THE VOLUME PERCENTAGE OF CELLS AND PLASMA UPON A SINGLE PERMANENT RECORD

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AFTER a thorough review of the subject of sedimentation of blood cells, it was realized that of the many methods of recording the phenomenon, none accurately measured all phases of the rate of descent of the line of demarcation between cells and plasma. One method,[1] based upon the fact that light passes through plasma and not through cells, suggested the possibilities of photography as a means of automatically recording the rate.

With the cooperation of F. W. Davis, of the Photography Department of Ohio State University, various photographic methods were tried, until finally it was decided to attempt to build an instrument that would transfer an image of a very narrow vertical portion of a blood-filled tube through a photographic lens onto sensitized paper attached to a revolving drum.

Fig. 1 illustrates the mechanics as applied to this principle:

A represents the light source, a 75 watt tubular showcase bulb with a long filament to illuminate a Wintrobe hematocrit tube[2] fairly uniformly from top to bottom.

B is a glass-walled chamber or condenser through which water is run to eliminate the heat of the light source and cool the chamber containing the blood sample to well below room temperature. There is a pane of glass between *A* and *B* establishing an air chamber between the bulb housing and water jacket. This further aids in eliminating the heat of the light source.

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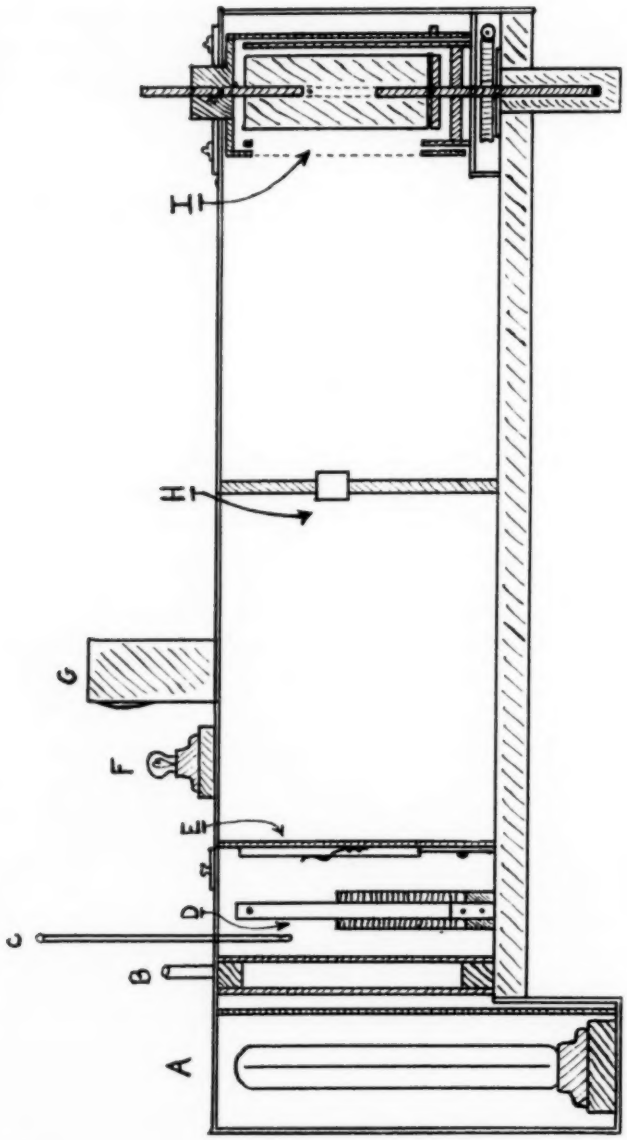

Fig. 1.

C is a thermometer inserted in the chamber containing the blood sample.

D represents a thermostatically-controlled nonglowing heating element incorporated to maintain in the chamber containing the blood tube any temperature that may be desired above that produced by the cooling system. At present the thermostats are regulated to hold the temperature at approximately 37° C. while the apparatus is in use. The heating element and thermostat are so constructed as not to interfere with the proper illumination of the Wintrobe tube.

E is a blood tube holder containing a vertical slit about 0.003 of an inch wide. This holder is perpendicular to the flat table top upon which it is built. With the aid of water levels and adjustable screws in the legs, the table is leveled.

To minimize vibration, the table is placed on a cement floor. To further aid in minimizing vibration, the motor which activates the drum is mounted upon rubber and also fastened to the cement floor.

F is a red pilot light for assistance in adjusting the thermostat.

G is an electric time switch clock.

H represents a photographic lens which transfers in proper focus an image of a very narrow portion of the Wintrobe tube onto photographic paper on drum *I*.

I is a drum for holding sensitized paper. This drum is perpendicular to the flat table top and made to travel at a predetermined slow speed by means of a motor and reducing gears. The speed of the drum may be altered if desired, but at present it is 0.92 r.p.h. This speed allows for a recording of sedimentation for one hour with additional space left on the sensitized paper to photograph the sample after centrifugation. Around the drum is a housing and shutter. These are so constructed that the sensitized paper may be placed upon the drum in a darkroom. The portion of the apparatus containing the sensitized paper (drum, housing, and shutter) may then be transferred to the unit in daylight and the shutter opened from outside of the unit. The apparatus from the blood tube holder to and including the drum is light tight and the remaining anterior portion can be made so at will if the instrument is to be used in a darkroom.

Three such units have been built and have produced very gratifying results. Although designed to more accurately establish "normals and physiological variations" in the blood of equines, the apparatus is sufficiently flexible to make its use possible with human blood or that of other animals.

Fig. 2 illustrates the results obtained from a half-hour exposure of horse's blood and a one-minute exposure of the same sample after centrifugation.

DD' is the top of the dark heavy line and represents the bottom of the column of blood. The length of *DD'* (or *AA'*) represents the time of exposure.

AD represents the height of the column of blood (100 mm.) at the start of the measurement when the cells are dispersed throughout the plasma.

The dark area *ABCA'* represents the exposed area of the graph brought about by the increasing amount of light passing through the plasma as the cells settle out.

The light area *ABCD'D* represents the unexposed area brought about by the presence of cells through which the light cannot pass.

AB represents the beginning and gradually accelerated descent of the line of demarcation between cells and plasma.

BC represents the gradually retarded descent of the line of demarcation between the cells and plasma brought about by the gradually increasing effect of heaping of cells in the lower portion of the tube.

B represents the theoretical point of change between the gradually accelerated descent (*AB*) and the gradually retarded descent (*BC*) of the line of demarcation between cells and plasma.

Sigmoid curve *ABC* then represents a graphic record of the descent of the line of demarcation between cells and plasma for a given time.

The small light horizontal lines in area *ABCA'* represent the millimeter divisions on the tube.

The small vertical lines in area *ABCA'* occur at intervals of approximately 1.0174 minutes as the apparatus is now run.

The narrow dark vertical lines preceding and following exposure of the sedimenting and centrifuged sample are made by slight exposure of the sensitized paper when the tube holder is empty.

UW represents the height of the column of blood (100 mm.).

UV represents the height of the column of plasma after centrifugation (volume percentage of plasma).

VW represents the height of the column of cells after centrifugation (volume percentage of cells).

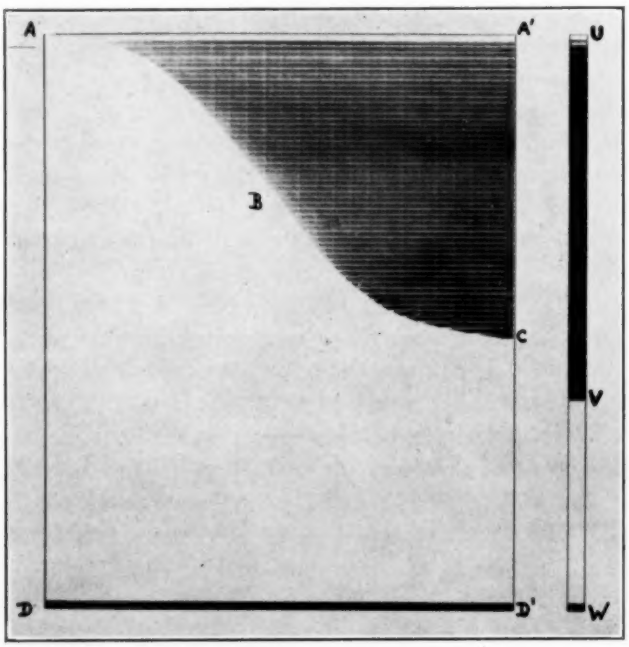

Fig. 2.

This mechanical means of measuring and recording cellular sedimentation is thought to embody the following advantages over previous methods:

1. It measures all phases of the sedimentation phenomenon as it occurs in the Wintrobe hematocrit tube.
2. It measures the rate of sedimentation continuously and automatically.
3. The record is free from personal error in measuring.
4. The record is permanent.
5. Volume percentages of cells and plasma for the same sample of blood are permanently incorporated upon the same record as the sedimentation rate. If desired one may alter the ratio of formed elements and plasma to a previously decided upon standard volume and photograph the corrected sedimentation rate and volume percentages of cells and plasma for comparison with the original sample.

6. Time heretofore consumed in periodically reading the position of the line of demarcation between cells and plasma can be used for other useful laboratory examinations.

7. More than one unit facilitates the comparison of more than one sample at the same time. This fact is a great asset in problems similar to the one now being conducted involving "standardization" of technique and establishment of "normals and physiological variations."

8. If one desires an accurate permanent record of the standardization of a centrifuge as to speed and time necessary to completely pack the formed elements of blood in the Wintrobe hematocrit tube, one needs only to stop the centrifuge at intervals and photograph the tube in the unit, repeating the procedure until no further packing occurs.

9. To standardize a shaking device for the proper agitation of blood samples prior to sedimentation determinations, the apparatus may be used for accurate permanent recordings.

10. It takes little thought to find uses for the apparatus in many phases of laboratory work other than in the field of hematology.

The apparatus described was manufactured for the author by W. F. Sipes, of Columbus, Ohio.

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THE USE OF AIR-COOLED CONDENSERS IN BLOOD CHOLESTEROL DETERMINATIONS*

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IN THE determination of blood cholesterol by methods employing the Liebermann-Burchard reaction, contamination of the chloroform extract of the blood with water should be carefully avoided, otherwise erroneous values will be obtained. In many clinical laboratories Lieboff tubes and water-cooled condensers are used in preparing the chloroform extract. Recently, some rather expensive modifications of this apparatus have been put on the market which permit moisture that forms on the outside of the condenser to trickle into the tube and thus to interfere with proper extraction or with subsequent color development. This usually occurs only when the tap water is very cold, or when the relative humidity in the laboratory is high. The difficulty is readily overcome by using air-cooled condensers, the cost of which is also negligible. Pyrex glass tubing, about 30 inches long and one-half inch outside diameter, will serve the purpose. The end, which passes through the cork fitting into the Lieboff tube, is drawn out somewhat. From the standpoint of simplicity and economy, as well as reliability, this arrangement leaves little to be desired.

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DETERMINATION OF HIPPURIC ACID IN URINE*

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QUICK some time ago recommended the estimation of hippuric acid that is excreted in the urine after the ingestion of benzoic acid as a test for liver function.[1] The test has two merits: it is simple and it gauges a well-defined function of the liver, i.e., its ability to conjugate benzoic acid with glycine. When the method came into use in this laboratory for various studies, the analytical technique described by Quick proved to be unsatisfactory, as it yielded inaccurate and erratic results. Quick determines either gravimetrically or volumetrically the amount of hippuric acid which crystallizes out upon acidification of the urine. Aware of the fact that the substance does not separate out completely, Quick adds to the amount of the hippuric acid found 0.33 gm. for each 100 c.c. of mother liquor. This method of correction implies the assumption that 100 c.c. of urine retain at ordinary temperatures 0.33 gm. of hippuric acid in solution and that all in excess of this amount crystallizes out.

Our experiments did not bear out this assumption. We found, namely, that the solubility of hippuric acid in urine at 22° to 26° C. is in the first place considerably greater than 0.33 per cent, and in the second place that it shows substantial variations with different urine specimens. In the experiments which disclosed this fact, known quantities of pure hippuric acid were dissolved in measured volumes of urine samples and subsequently recovered with strict observation of the directions given by Quick. As shown in Table I, the solubility of hippuric acid was in every instance much greater than 0.33 per cent; the lowest figure we found was 0.44, the highest 0.62 gm. per 100 c.c. of urine. To use for correction the average of values which spread over such a wide range would be too crude a procedure. The high degree of solubility of hippuric acid, furthermore, greatly limits the usefulness of the method, since if a urine contains no more than 0.5 to 0.6 gm. of the substance per 100 c.c., none of it separates out after acidification, and the urine passes analysis as containing no hippuric acid at all; likewise, if the hippuric acid content is 0.25 to 0.30 gm. per 100 c.c., it eludes detection even if the urine is concentrated to one-half of its original volume.

In view of the merits of the test we attempted to increase the accuracy of the analytical technique. Our purpose was substantially to diminish, and at the same time to render more uniform, the solubility of hippuric acid in various

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TABLE I

SOLUBILITY OF HIPPURIC ACID IN URINE

GRAMS HIPPURIC ACID ADDED	VOLUME URINE C.C.	GRAMS HIPPURIC ACID RECOVERED	GRAMS HIPPURIC ACID HELD IN SOLUTION PER 100 C.C. URINE
0.5	50	0.211	0.578
1.0	50	0.720	0.560
1.0	50	0.687	0.626
1.0	100	0.506	0.494
1.0	100	0.394	0.606
2.0	100	1.560	0.440
1.0	150*	0.759	0.482
1.0	150*	0.737	0.526

*Evaporated to 50 c.c. before analysis.

samples of urine, without making the method unduly tedious for use in clinical diagnostic work. A simple step, i.e., saturation of the urine with sodium chloride, produced the desired conditions. As the figures in Table II show, at ordinary room temperatures 100 c.c. of urine in which 30 gm. of sodium chloride is dissolved, holds in solution only 0.110 to 0.137 gm. of hippuric acid. Thus its solubility has been cut to nearly one-fifth, and the variations have become relatively slight; the difference between extreme figures has been reduced from (626–440) 186 to (137–110) 27 mg. per 100 c.c. Hence the average of the solubility figures, 0.123 gm. per 100 c.c. of urine, can be adequately used as a correction by addition to the amount of hippuric acid which had separated out from the acidified urine. This modification allows the analysis of 150 c.c. and even 200 c.c. of urine without preliminary concentration, whereas the maximum volume permitted by Quick is 100 c.c. In addition, the time required for the complete crystallization and separation of hippuric acid also is considerably shortened.

TABLE II

SOLUBILITY OF HIPPURIC ACID IN URINE AFTER SATURATION WITH SODIUM CHLORIDE

GRAMS HIPPURIC ACID ADDED	VOLUME URINE C.C.	GRAMS NaCl ADDED	GRAMS HIPPURIC ACID RECOVERED	GRAMS HIPPURIC ACID HELD IN SOLUTION PER 100 C.C. URINE
0.5	100	30	0.377	0.123
0.5	100	30	0.370	0.130
1.0	50	15	0.920	0.120
1.0	100	30	0.880	0.120
1.0	100	30	0.870	0.130
1.0	100	30	0.890	0.110
1.0	100	30	0.886	0.114
1.0	150	45	0.816	0.123
1.0	150	45	0.795	0.137
			Average	0.123

The analytical procedure is carried out as follows: Measure the volume of the urine specimen. If it is above 150 c.c., add a few drops of glacial acetic acid and evaporate to a volume not in excess of 150 c.c. Add 30 gm. of sodium chloride per 100 c.c. of urine and heat with shaking until the salt is dissolved. Cool the solution to about 15°-20° C. by immersion of the flask into ice-cold water, add 1.2 c.c. of approximately 10 N sulfuric acid, and scratch the sides

of the flask with a glass rod to enhance the crystallization of the hippuric acid. (This apparently trivial step is important.) Allow to stand for fifteen minutes in the cold water bath, then filter through a Hirsch funnel (diameter of perforated plate 47 mm.), using moderate suction. Wash the precipitate with chilled 30 per cent sodium chloride solution from a wash bottle, using the washing fluid first to rinse the flask in which the precipitation has been performed. It is not necessary, however, to transfer the precipitate to the filter quantitatively. The precipitate is adequately washed when the washing fluid is free of sulfuric acid. Transfer the funnel with its contents onto the flask that still contains some of the hippuric acid crystals, and rinse the filtered hippuric acid into it by dissolving it in hot water; to this end use a fine tipped wash bottle. All of the hippuric acid is now in the flask in which it had been precipitated; heat until all the hippuric acid particles adhering to the flask dissolve, and titrate while hot with 0.5 N sodium hydroxide, with phenolphthalein as an indicator.

CALCULATION

The number of cubic centimeters of 0.5 N sodium hydroxide used for neutralization is multiplied by 0.072, which gives the amount of sodium benzoate in grams from which the hippuric acid was derived. If to this amount one adds the correction for the solubility of hippuric acid (0.123 gm. per 100 c.c. of urine) in terms of sodium benzoate, i.e., 0.123 by 0.804 = 0.10 gm. sodium benzoate, one obtains the sodium benzoate value of the specimen with a maximum error of ± 10.5 mg. per 100 c.c. *Example:* The volume of the urine specimen analyzed (excreted during one hour after the ingestion of 4 gm. of sodium benzoate) was 134 c.c. The amount of sodium chloride added was 40 gm. (more accurately 1.34 by 30 = 40.2 gm.). The precipitated hippuric acid required 11.2 c.c. of 0.5 N sodium hydroxide for its neutralization. This corresponds to 11.2 by 0.072 = 0.806 gm. sodium benzoate. The correction for solubility would be 1.34 by 0.10 = 0.134 gm. sodium benzoate. The sodium benzoate value of the specimen equals 0.940 gm. The probable error is ± 10.5 × 1.34 = ± 14 mg. sodium benzoate, which corresponds to 1.4 per cent of the quantity determined.

Expressing the amount of the hippuric acid excreted in terms of sodium benzoate simplifies the visualization of its relationship to the amount of the sodium benzoate ingested. Thus our example shows that in one hour after the ingestion of 4 gm. of benzoate, 0.937 gm., or 23.4 per cent, of it had been excreted.

Occasionally one encounters urine specimens that are markedly bile-tinged or dark-colored. The analysis of such urines is difficult because the hippuric acid clogs the filters due to the admixture of colloids. A further difficulty in such cases is caused by the fact that the final hippuric acid solution is too highly colored and as a consequence the end point of the titration is difficult to determine. This difficulty can be easily overcome by the following procedure: Add 0.3 gm. of acid-washed norit per 100 c.c. of urine, and

boil the mixture for about one minute. After cooling, filter by suction on a Hirsch funnel, and wash the norit residue with a small quantity of hot water; measure the volume and proceed as outlined.

If the urine samples contain protein, this must be completely removed before the determination of the hippuric acid content. To this end, measure the volume of the sample, add 5 c.c. of 20 per cent copper sulfate solution, mix, then with agitation introduce 5 c.c. of N/1 sodium hydroxide per 100 c.c. of urine. In exceptional cases with very large albumin content, double these amounts. Shake and heat to near the boiling temperature. After cooling, filter through a good grade of filter paper into a graduated cylinder and record the volume of the clear filtrate. For the rest the procedure is the same as for normal urines, but for the fact that the urine was diluted with the protein precipitants and that only an aliquot portion was used for analysis. This must be taken into account in the calculation of the results.

SUMMARY

The analytical technique of Quick for the determination of hippuric acid in urine is inaccurate. A modification is proposed which renders the method sufficiently accurate for clinical diagnostic work while retaining its original simplicity. Directions are described for the preliminary treatment of urines which contain bile, dark coloring matter, or albumin.

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A DIFFERENTIAL MANOMETER METHOD FOR THE MEASUREMENT OF BLOOD FLOW*

HAMPDEN LAWSON, M.D., PH.D., AND J. P. HOLT, M.D., LOUISVILLE, KY.

THE principle of the Venturi meter was used in 1926 by Daly, and with modifications in 1927 by Wagoner and Livingston, for the measurement of blood flow. In neither case did the differential manometer employed permit a direct graphic recording of flow.

In order to obtain such a record without resorting to optical methods, a differential manometer was constructed which would permit the taking of direct mechanical records on smoked paper. In principle, the manometer differs very little from the familiar Pachon capsule, except that the membrane between the pressure chambers is double, which permits sealing each chamber airtight. Each chamber thus becomes a simple tambour. When the membranes have been attached (condom rubber) the chambers are bolted together, membrane to membrane, with the recording lever sandwiched between,

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cemented to one or both membranes with rubber cement. The lever passes out of the capsule through a shallow notch cut in the rim of both tambours (Fig. 1). In use, both chambers and all lead tubes are completely filled with 5 per cent sodium citrate solution.

A defect in all rubber membrane manometers to which attention has frequently been directed is the inconstancy of the membrane. This defect is present in a differential manometer of the type described, where the restoring force is the elasticity of the rubber membrane. We have observed slight changes in the calibration curve of these manometers for the first half hour or so following their completion, which we attribute in part to drying of the rubber cement, and in part to changes in the elasticity of the membrane. If the tambours are allowed to dry thoroughly, however, after the membranes have been applied, and two or three maximum deflections in both directions are produced, the manometer gives constant deflections thereafter for a period of one or two days, in rubber tube hydraulic systems. Since replacement of the membranes requires only a few minutes, it has been our practice to start each day's work with fresh membranes, allowed to dry for at least a half hour, and maximally stretched two or three times.

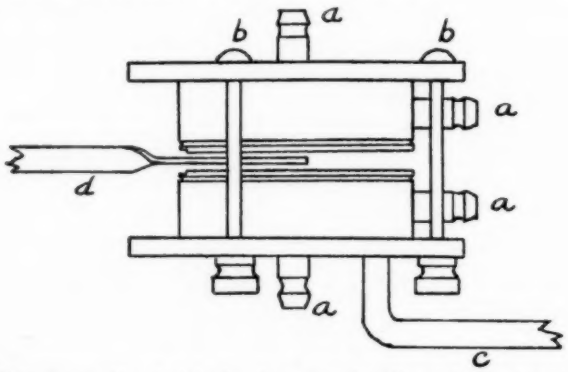

Fig. 1.—Semidiagrammatic sketch to show construction of membrane manometer. The two halves are slightly drawn apart to show insertion of the lever *d* between the membranes. The lever is a thin strip of phosphorbronze, about 3 mm. wide, which passes through a shallow notch cut in the rim of each chamber, to avoid binding. The membrane (not shown in the sketch) passes over this notch and is tied in its groove on each chamber. The bolts *b* are loosened to replace the membranes, and tightened when the manometer is in use. The apposed rims of the two chambers are made smooth and broad to avoid cutting the membranes when the bolts are tightened, except where the lever passes out of the capsule. Here the notched rim of each tambour is filed to a dull knife edge. Three bolts suffice, equally spaced about the circumference. Each chamber has two metal nipples *a* for connecting rubber tubing, to facilitate washing through with citrate solution. To wash out air bubbles in the lower chamber, the manometer is rotated about the supporting rod *c* until the wash-out tube in the lower chamber is uppermost. The inside diameter of each chamber is 3 cm., the inside depth 1.2 cm.

In order to apply the Venturi principle without using anticoagulants, Wagoner and Livingston constricted the aorta with an oblique ligature at the origin of a sizable branch, which was cannulated and led to one limb of the manometer. The other limb of the manometer was connected by cannulation with an artery originating above the constriction. We found it very difficult to place a dependable ligature so as to produce this type of constriction. In trials on animals and on rubber tube models, the pressure difference for a given flow was found to be at least as great if the lower limb of the manometer was connected below the constriction rather than in it. This procedure was, therefore, followed in all subsequent work.

Merriman (1916) gives the fall in head below a constriction in a tube as:

$$H = \left(\frac{a}{a_1} - 1\right)^2 \frac{v^2}{2g}, \text{ where}$$

H is the loss of head, a the cross-sectional area of the tube, a_1 the area of the constriction, and v the velocity in the tube. Thus, with a constant constriction, the fall in head below the constriction is a function of the velocity of flow. No attempt was made in this work to produce a standard constriction in the artery. A small Gaskell clamp, with one limb cut through so that it could be swiveled into place on the vessel, was tightened down until pressure on the distal side fell 3-5 mm. Hg. This was sufficient difference in pressure to give about half of the full deflection from the zero point of the manometer. The constriction, as well as the manometer, was calibrated at the end of each experiment, when absolute values for flow were required, by opening the

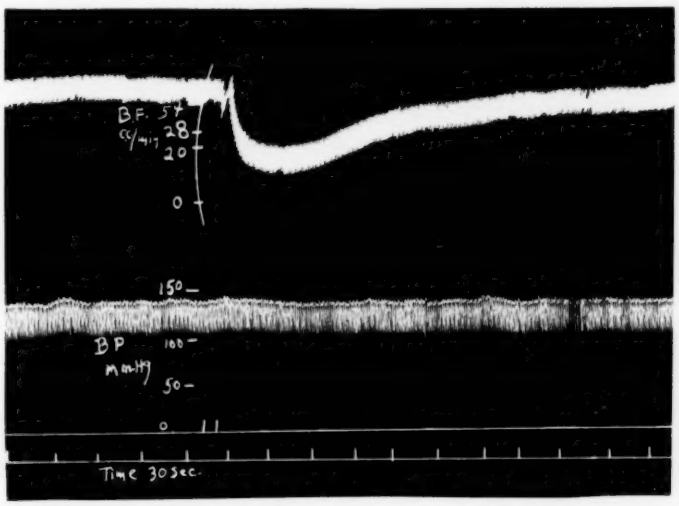

Fig. 2.—Records, from above downward: (1) blood flow into hind limb of barbitalized dog; (2) carotid blood pressure (mercury manometer); (3) signal and base line for carotid pressure; (4) time in thirty-second intervals. Between signals 1 c.c. epinephrine 1:50,000 was injected into the femoral artery through the central stump of the ligatured internal iliac artery. Note the immediate transitory reduction in blood flow during the injection, due to the rise in pressure in the femoral artery from the volume of fluid injected. This is followed by the typical constrictor effect of epinephrine.

artery below the lower limb of the manometer and measuring directly the flow required to produce manometer deflections observed in the experiment. As a rule, each deflection was checked against flow at least twice. In the second calibration blood pressure was usually considerably lowered by hemorrhage, yet the two deflections for a given flow checked with an error of not more than 3 per cent. This confirms observations on artificial systems that the calibration of the manometer is independent of the head of pressure over a rather wide range.

In order to measure flow into an organ by this method it is, of course, necessary that all blood flowing past the constriction enter the organ being studied. For flow into the hind limb of the dog, we have placed the constriction on the aorta just above its bifurcation into common iliacs, cannulated one

iliac (after tying all intervening branches), and tied all branches on the contralateral iliac as far distally as the inguinal ligament (Fig. 2). Clotting in the cannula was less frequent if the stump of iliac to be cannulated was left long.

If it is assured that pressure just above the constriction rises and falls exactly with carotid pressure, the upper limb of the manometer may be connected with the carotid. In the work which we have done on blood flow through the leg as influenced by local effects, there is good ground for believing this to be true. In our heavily barbitalized or etherized dogs the difference in pressure in the carotid and the femoral artery, before constricting the aorta, was never greater than 1 mm. Hg, and remained constant through minor spontaneous changes in carotid pressure (mercury manometer connected with remaining carotid) such as occurred in the course of a half-hour's observation. Furthermore, even maximum changes in flow in one leg do not appear to affect the lateral pressure in the lowermost segment of the aorta under these conditions, since the differential was unaffected by completely occluding the remaining femoral artery. It was, therefore, concluded that, for our purposes, carotid pressure is a sufficiently accurate measure of pressure throughout the aorta down to the constriction. It is theoretically possible, however, that under some experimental conditions, such large deflections of flow may occur between the carotid and the lowermost segment of the aorta as to invalidate this technique. Where such deflections of flow may occur, the upper limb of the manometer should, for the sake of simplicity, be connected just above the constriction.

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THE QUANTITATIVE DETERMINATION OF URINARY ESTROGENIC HORMONE (BIOLOGICAL METHOD)*

A Consideration of the Technique, Animal Colony, and Laboratory Personnel

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INASMUCH as no chemical method of measuring estrogenic hormone has proved practical for the urine of nonpregnant women we must still rely upon a biological procedure for this purpose. Since the chemical identity of the estrogenic hormone in the urine of nonpregnant women has not, as far as I know, been accurately determined, it is safest to speak of such hormone as having estrogenic potency equivalent to a chemically pure hormone of known weight, preferably estrone. The value of determining the "free" and "combined" estrogenic hormone proportions in the urine of pregnant women, and possibly also in the urine of nonpregnant women, has been demonstrated.[1-3]

The procedure to be outlined and discussed here has been developed from seven years of personal experience, with most of the described methods of biological assay, a knowledge of chemical methods, and from particular attention paid to the standardization of values obtained. Nearly a thousand urinary extracts have been prepared, of which 550 have been completely assayed. The procedure is described in two sections, the first dealing with the preparation of animals and the second with the preparation of urinary extracts. The method of preparing animals for testing is original and, except for a change in the grouping of animals, is identical with an earlier report.[4] No part of the technique on the preparation of extracts is original. It is a combination of the best features from several methods. The procedure can be applied to the urine of pregnant as well as nonpregnant women, men, and children.

PROCEDURE

A. *The Preparation of Animals.*—On a Monday each of a hundred 4-month-old female mice is castrated through a short dorsal incision, after which a skin clip is applied to allow healing of the wound. One week later, the skin clips are removed and each mouse is given a subcutaneous injection of 2 gamma estrone in 0.2 c.c. olive oil. On Monday of the next week each mouse is given 0.2 gamma estrone in 0.2 c.c. olive oil, and on the third day following a wire loop scraping of the vagina of each mouse is examined. Only those animals exhibiting complete vaginal cornification are set aside as eligible test animals

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for unknown urinary extracts to be injected four days later, three weeks from the date of castration. The principle evolved here is one of overstimulating the animals. The dose given at the first injection (2 gamma) will cause every animal to go into estrus, consequently no examination of vaginal smears need be made. The dose of estrone given in the second injection (0.2 gamma) has been found to be the best minimally effective dose of estrone to which the most sensitive of animals will react. Early experiments revealed that while an animal would occasionally react to 0.1 gamma estrone, the percentages of animals responding to this small dose and the frequency with which the same animal would respond to the same small dose were so slight that it did not seem practicable to follow this earlier procedure. Admittedly then, the acceptance of 0.2 gamma estrone as the minimal effective dose introduced a possible error of 100 per cent at the outset, but this is minimized by the proper spacing of dose levels and by the use of three controlled animals for each test dose.

Animals not exhibiting estrus after the administration of the first 0.2 gamma estrone dose are overstimulated again with 2 gamma and given a second trial with 0.2 gamma estrone. To those animals still not responding to the minimal dose, the overstimulating procedure is repeated a third time before finally discarding nonresponsive animals. All injections, whether they be overdoses, minimal doses, or test doses of unknown extracts, are given routinely on Monday of each week. Vaginal smears of test animals only are examined Monday morning before injection, Wednesday morning and afternoon, Thursday morning and afternoon, and Friday morning. Full vaginal cornification in the animals under test, as revealed in the Thursday morning smear, is the only criterion of a positive reaction, and this is the same end point obtained when the known standard is used. The other smears from the animals under test are useful in checking, for evidence of persistent estrus and for error in castration.

After an animal has been used for testing an unknown extract, it is given the minimal dose of estrone a week later, provided it went into estrus with the unknown extract, or the overdose followed by the minimal dose if it did not go into estrus with the unknown. *Every animal used for testing must have responded in full estrus to the known standard minimal dose of estrone one week before.*

Three animal bins, each 1 foot by 2 feet by 1 foot, are maintained for each series of 100 castrated test mice. The first bin holds animals under test for the week, the second holds animals injected with 0.2 gamma estrone, and the third holds animals injected with 2 gamma estrone. Vaginal smears must be obtained from the animals in the first bin of each series on Monday, Wednesday, Thursday, and Friday; from the animals in the second bin of each series Thursday morning only; and not at all from the animals in the third bin of each series. Two or three bins are maintained for the newly castrated animals and as they respond for the first time to the minimal dose of estrone they are given numbers and used to fill in, as replacements, the missing numbers in any of the test animal series.

B. *The Preparation of Urinary Extracts.*—My method of estrogenic hormone extraction combines important features of several other methods.[5-9] Since it is suspected that sudden hormone excretion may occur at times during a period of a few hours or less, it is advisable always to require a complete twenty-four-hour urine specimen when an assay is to be done. This is particularly necessary when one wishes to demonstrate the daily excretion of hormone by any sort of a graph. I believe that a twenty-four-hour specimen of less than 800 c.c. with a specific gravity below 1.010 cannot be considered

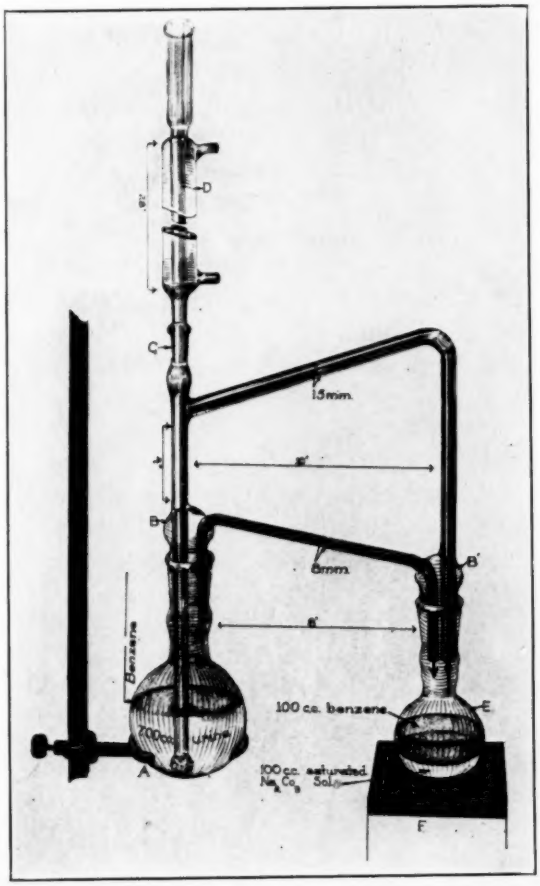

Fig. 1.—Continuous extraction apparatus (modified from Kurzrok and Ratner[6]). *A*, 1000 c.c. flask; *B* and *B'*, standard groundglass coupling No. 35; *C*, standard groundglass coupling No. 15; *D*, water cooled reflux condenser; *E*, 500 c.c. flask; *F*, steam bath.

complete. The specimen should be collected in a jar containing about 2 c.c. of toluol, and if possible kept on ice during the period of collection. Toluol is a nontoxic preservative and does not interfere with quantitative gonadotropic hormone procedures. Any amount of estrogenic hormone that may go into the toluol preservative is automatically transferred to the "free estrin" extract. A maximum time limit, preferably twelve hours, should be adhered to between the time the collection of a specimen is completed and the time the

extraction of free estrin is started. This is of greatest importance with reference to the urine of pregnant women where slight bacterial hydrolysis is apt to occur in the urine on standing.

Each twenty-four-hour urine specimen is made up to 1,200 c.c. with distilled water if it does not already exceed that volume. Of this, 700 c.c. are used for estrin assay, and 500 c.c. set aside for gonadotropic hormone assay. The 700 c.c. portion is put directly into a continuous extraction apparatus (Fig. 1) and extracted with benzene for twenty-four hours. Chloroform, ether, ethyl acetate, and benzene have been tested for their efficiency in extracting internationally standardized estrone from aqueous solution. Of these solvents benzene has been found to be the most efficient. The collecting flask contains 100 c.c. of a saturated sodium carbonate solution as a wash for the benzene extract containing the hormone. Saturated sodium carbonate solution has been found not to remove estrogenic hormone from benzene solution. The latter is then transferred to a separatory funnel and washed with 100 c.c. of 2.5 per cent hydrochloric acid, saturated with sodium chloride, to remove any trace of alkali. A small amount of olive oil is added to the benzene solution and the benzene removed by steam heat. The final extract is made up to 7 c.c. with olive oil, thus making a 100-fold concentration of urinary estrin in its "free" state.

One hundred cubic centimeters of concentrated hydrochloric acid are next added to the 700 c.c. portion of urine and the mixture autoclaved for one and one-half hours under 15 pounds pressure at 120° C. After autoclaving, the urine is re-extracted with benzene for twenty-four hours, and the extract washed and evaporated as before and put into olive oil as a 100-fold concentration of urinary estrin in its "combined" state.

The olive oil extracts can be kept indefinitely without danger of deterioration. Assays have been carried out on the same extracts as long as two years apart, with identical values obtained.

Injections are made according to the form sheet in Table I and corrections for the twenty-four-hour volume calculated. Values for less than 4.0 gamma of estrone per liter of urine are not determined. When 4.0 gamma per liter cannot be demonstrated, the value is recorded as negative.

NORMAL VALUES FOR WOMEN

An absolutely accurate and full statement of normal values cannot yet be made due to the difficulties encountered in carefully collecting and assaying complete, consecutive twenty-four-hour urine specimens from truly normal individuals. However, it is safe to say that a normal woman excretes from 4 to 30 gamma of estrone-equivalent per day, except during the time she is menstruating, when the amount is usually not demonstrable. The excretion is greater during the second half of the intermenstruum. For one or two days at midintermenstruum there is normally a peak excretion that may amount to as much as 200 gamma. This peak is supposedly associated with ovulation. A consideration of the mechanism involved in ovulation and associated hormone excretion in a normal woman has been given previously.[2] A premenstrual rise in estrogenic hormone excretion is slight and inconstant.

TABLE I

URINARY ESTROGEN

FREE
COMBINED
24-HOUR VOLUME

TEST NO.
PATIENT
DATE

		ANIMAL NO.	48-56 HOURS	72-80 HOURS	96 HOURS	
Extract (Whole)						
4.0/L	Date injected:	1				
	Dose: 0.5 c.c.	2				
		3				Reaction:
6.6/L	Date injected:	1				
	Dose: 0.3 c.c.	2				
		3				Reaction:
10.0/L	Date injected:	1				
	Dose: 0.2 c.c.	2				
		3				Reaction:
20.0/L	Date injected:	1				
	Dose: 0.1 c.c.	2				
		3				Reaction:
Extract (1:10 dilution)						
40.0/L	Date injected:	1				
	Dose: 0.5 c.c.	2				
		3				Reaction:
66.6/L	Date injected:	1				
	Dose: 0.3 c.c.	2				
		3				Reaction:
100.0/L	Date injected:	1				
	Dose: 0.2 c.c.	2				
		3				Reaction:
200.0/L	Date injected:	1				
	Dose: 0.1 c.c.	2				
		3				Reaction:
Extract (1:100 dilution)						
400.0/L	Date injected:	1				
	Dose: 0.5 c.c.	2				
		3				Reaction:
666.6/L	Date injected:	1				
	Dose: 0.3 c.c.	2				
		3				Reaction:
1000.0/L	Date injected:	1				
	Dose: 0.2 c.c.	2				
		3				Reaction:
2000.0/L	Date injected:	1				
	Dose: 0.1 c.c.	2				
		3				Reaction:
Extract (1:1000 dilution)						
4000.0/L	Date injected:	1				
	Dose: 0.5 c.c.	2				
		3				Reaction:
6666.6/L	Date injected:	1				
	Dose: 0.3 c.c.	2				
		3				Reaction:

------ gamma per 24 hours
(Calculated value estrone-equivalent)

Throughout the cycle of a normal woman the urinary estrin has been found to be in its combined fat-insoluble state. Only by extraction and assay of consecutive complete twenty-four-hour urine specimens from the same individual may the transient excretion of free estrin be demonstrated at mid-intermenstruum and again just premenstrually.

An excretion of less than 4 gamma estrone-equivalent per liter of urine seems to be definitely abnormal for a normal adult woman not bleeding. Since more than this amount has been found in some cases of persistent amenorrhea and natural menopause and since this amount has never been demonstrated in a patient whose ovaries have been removed, I believe that 4.0 gamma is an acceptable excretory level below which ovarian inactivity or absence of estrogenic hormone elaboration is quite definite.

LABORATORY HELP AND THE ANIMAL COLONY

Equipment and personnel for a laboratory to carry out routine hormone assays deserve much consideration. A statement as to the practicability of such a laboratory is not ventured. Two full-time technicians and a full-time animal caretaker are the minimum for the required number of persons devoting all of their time to this work.

An animal colony of not less than 1,000 mice should be maintained. Approximately 400 castrated adult female mice on hand at all times are necessary for the routine testing of twelve twenty-four-hour urine specimens each week. To maintain this number of animals for testing, it has been found necessary to castrate approximately 100 animals every three weeks.

The size of the animal colony advised is sufficient to allow almost an unlimited number of Aschheim-Zondek pregnancy tests and quantitative chorionic gonadotropic hormone assays.

A review of figures collected over a year's time reveals that the exposure of 40 adult female mice to males each week (1 male to 8 females) has been productive of an average of 17 litters of newborn mice per week. Too low a room temperature seems to have been the greatest single factor responsible for low fertility. This manner of breeding supplies an average weekly yield of 80 young females which can be used for pregnancy tests or quantitative gonadotropin testing when 21 days old. Those not used at 21 days maintain the strength of the colony for estrogenic hormone tests and for further breeding.

All but an extremely small percentage of the newborn males are destroyed when 2 or 3 days old. This allows a standard litter size of four to five young females to each mother. With the advent of a good rodent test for male sex hormone, the specifications for a laboratory described herein can be readily adjusted to supply animals for the quantitative determination of urinary androgen.

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FULMINATE-FERRICYANIDE REAGENT*

With a Note on a Color Reaction for the Fulminates

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THE utility of direct titrations with ferricyanide solutions has been somewhat impaired by their pale color. Consequently, their decolorization to ferrocyanide is an end point difficult to read. We have searched for an internal indicator to be used with the alkaline ferricyanide reagent for the time method of blood sugar determination of Hawkins and Van Slyke,[1] as well as for the ferricyanide reagent employed in direct uric acid titration by the method of Barnard.[2] Only accidentally was it discovered that the fulminates (those of silver, sodium, and mercury were investigated in this connection) strike with ferricyanide, in neutral or alkaline solution, a deep rose red color which appears characteristic for the fulminates. This coloration is evidently the result of the formation of an addition compound since the oxidant nature of the ferricyanide is not vitiated. The titer of the fulminate-ferricyanide solution is equal to that of untreated ferricyanide solution as determined by titration of uric acid solutions.

The reagent is prepared by the addition of mercuric fulminate to potassium ferricyanide solution. It appears to keep indefinitely.

Preparation of Mercuric Fulminate Suspension.—Ten grams of metallic mercury are dissolved in 10 c.c. of concentrated nitric acid, and the resulting solution is poured into an equal volume of 90 per cent ethyl alcohol in a large Erlenmeyer flask. The reaction, which is rather turbulent, is allowed to proceed under a hood. When complete, the supernatant fluid is carefully decanted and the grayish deposit is washed with several changes of distilled water until all traces of acid are removed. The fulminate is kept under water. The danger in its handling has been considerably overestimated.

Preparation of Centimolar Fulminate-Ferricyanide Reagent.—0.329 gram of crystalline potassium ferricyanide is dissolved in about 50 c.c. of distilled water. A few crystals of the moist mercuric fulminate are added, and the

*From the Laboratory of Therapeutics of the College of Medicine, University of Illinois, Chicago.

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reagent diluted to 100 c.c. The color intensity reaches its maximum in twenty-four hours, after which the reagent is ready for use. There should always be a slight excess of undissolved mercuric fulminate at the bottom of the container.

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1853 W. Polk Street

DEMONSTRATION OF THE BACTERIOSTATIC ACTION OF SULFANILAMIDE UPON HEMOLYTIC STREPTOCOCCI IN VITRO*

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FOLLOWING the important discovery of Domagk[1] of the therapeutic effectiveness of prontosil in experimental infections of mice with hemolytic streptococci, numerous articles have been published concerning various phases of this new form of chemotherapy of bacterial infections. It is beyond doubt that sulfanilamide and related compounds are effective also in the treatment of human infections due to hemolytic streptococci. In the first publication, Domagk emphasized that prontosil exerts its effectiveness only in vivo but not in vitro. In 1936, Colebrook and Kenny[2] reported that the serum of patients treated with prontosil retards the growth of hemolytic streptococci. In the same year, Colebrook, Buttle, and O'Meara[3] conclusively showed that sulfanilamide is bacteriostatic toward hemolytic streptococci; they found that small amounts of sulfanilamide (1:10,000) may delay the growth of hemolytic streptococci for several days, provided that a small number (30-50) of hemolytic streptococci were inoculated into the respective broths. When large numbers (30,000,000) of hemolytic streptococci were used, even a 1 per cent concentration of sulfanilamide failed to inhibit the growth of the organisms. Independently, Long and Bliss[4, 5] noted the bacteriostatic effect of sulfanilamide on hemolytic streptococci in vitro. Bliss and Long[6] furthermore demonstrated that a 1:1,000 diluted sulfanilamide lacked bactericidal effectiveness toward hemolytic streptococci suspended in broth or serum broth. The bacteriostatic effect of sulfanilamide in vitro toward small numbers of hemolytic streptococci was also corroborated by Osgood.[7] Mellon and Bambas[8] noted the marked bacteriostatic action of sulfanilamide upon hemolytic streptococci in spinal fluid of treated patients with streptococcal meningitis. It is important to state that the bacteriostatic action of a 1:1,000 diluted sulfanilamide is not demonstrable with all types of hemolytic streptococci. Bliss and Long[9] con-

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clusively showed that such amounts of sulfanilamide failed to retard the growth of group D Lancefield hemolytic streptococci. This finding is of considerable clinical importance. In this connection it is of interest to mention that Helmholz[10] found the urine of patients, treated with prontylin, bactericidal toward staphylococci and various gram-negative bacilli, but not toward enterococci.

It follows from this brief review of the literature that sulfanilamide is bacteriostatic toward certain strains of hemolytic streptococci. The demonstration of this bacteriostatic action, however, is a rather laborious procedure. The following is a description of a test tube method for the differentiation of sulfanilamide-susceptible and sulfanilamide-resistant strains of hemolytic streptococci. This method does not necessitate a dilution of the broth used for inoculation of the test medium nor a count of the number of microorganisms before and after treatment with sulfanilamide.

TECHNIQUE

Phenol red broth base (Difco) in the given amount was dissolved in neutralized distilled water; then 1 per cent soluble starch, 0.2 per cent agar, and 1 per cent maltose or dextrose, respectively, were added. The mixture was heated in a water bath until completely dissolved. Sulfanilamide powder was added to one part, the other part was used as a control. The sulfanilamide preparation used was prontylin, repurified for injection. After the sulfanilamide was dissolved by heating in a water bath, both broths were autoclaved for twenty minutes at 15 pounds pressure. The culture medium, referred to as phenol red broth and sulfanilamide phenol red broth, respectively, was always kept in the incubator at 37° C.

Various strains of hemolytic streptococci, isolated in this laboratory from human sources, were inoculated in phenol red broth containing sulfanilamide, as well as in control broth. About one loopful of sixteen to eighteen hours' plain infusion broth culture was used for inoculation. The inoculated culture media were incubated at 37° C. Growth in the control phenol red broth, which is accompanied by change of the indicator from red to yellow due to the acid production, was used as the standard and compared with growth and acid production in the sulfanilamide phenol red broth. The difference in growth was taken as an indication of the bacteriostatic action of sulfanilamide upon the respective strain.

RESULTS

In the first experiment, 10 different strains of hemolytic streptococci were inoculated into (1) phenol red broth, containing 0.8 per cent sulfanilamide, and (2) phenol red broth control. Table I gives the results obtained. Of the 10 strains tested, 6 (group 1) were completely or markedly inhibited in sulfanilamide phenol red broth. Growth in the controls occurred after five to twelve hours' incubation at 37° C. After sixteen hours' incubation at 37° C., one of the 6 strains grew out in sulfanilamide phenol red broth, although the growth was markedly less than in the respective control. The remaining 5 strains failed to grow out in sulfanilamide phenol red broth even when incubated for two weeks. The 6 strains tested in this series were found to be

fibrinolytic and to produce in 1 per cent glucose broth a pH above 5.0. The remaining 4 strains (group 2) were not or only slightly inhibited in 0.8 per cent sulfanilamide phenol red broth. Three of these strains were identified as hemolytic enterococci by their growth on 40 per cent bile agar, their capacity to reduce methylene blue and litmus in skimmed milk, by their production of a pH below 4.8 in 1 per cent glucose broth, and by their ability to ferment mannitol. The remaining strain of this group, which was not inhibited by sulfanilamide, produced a pH of less than 5.0 in 1 per cent glucose broth and lacked fibrinolytic activity. This latter strain was isolated from the urine of a patient with cystitis.

TABLE I

BACTERIOSTATIC ACTION OF 0.8 PER CENT SULFANILAMIDE UPON HEMOLYTIC STREPTOCOCCI IN PHENOL RED BROTH

	GROUP 1						GROUP 2			
Strains number	674	1179	1276	1372	1373	2521	23	26	2640	2887
Phenol red broth with 0.8 per cent sulfanilamide	−	−	−	−	+	−	++++	++++	+++	++++
Phenol red broth as control	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

− = No growth or acid production.
+ to ++++ = Various degrees of growth and acid production after sixteen hours' incubation at 37°C.

In order to test the action of various concentrations of sulfanilamide upon the growth of hemolytic streptococci, the following experiment was undertaken. Two strains of hemolytic streptococci (No. 1373 and No. 2521) were inoculated in phenol red broth containing various amounts of sulfanilamide: 0.8 per cent to 0.0000008 per cent (tubes a to g), respectively, and in phenol red broth without sulfanilamide as a control (tube h). The tubes were incubated at 37° C. The results are recorded in Table II. It may be seen from this table that both strains of hemolytic streptococci were inhibited in the presence of sulfanilamide. There were, however, quantitative differences to be observed. Strain No. 1373 failed to grow out in phenol red broth containing 0.8 per cent sulfanilamide; smaller amounts (0.08 per cent and less) were found ineffective, even after only eight hours' incubation at 37° C. On the other hand, strain No. 2521 was markedly inhibited in the presence of 0.000008 per cent sulfanilamide after eight hours' incubation; at this time, the

TABLE II

BACTERIOSTATIC ACTION OF VARIOUS AMOUNTS OF SULFANILAMIDE UPON HEMOLYTIC STREPTOCOCCI IN PHENOL RED BROTH

	CONCENTRATION OF SULFANILAMIDE															
STRAIN NO.	0.8%		0.08%		0.008%		0.0008%		0.00008%		0.000008%		0.0000008%		0	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
1373	−	−	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
2521	−	−	−	±	−	++++	−	++++	−	++++	+	++++	+++	++++	++++	++++

− = No growth.
+ to ++++ = Various degrees of growth and acid formation after: (a) eight hours at 37°C., (b) forty-eight hours at 37°C.

phenol red broth control showed profuse growth. After forty-eight hours' incubation at 37° C., however, growth occurred in phenol red broth containing up to 0.008 per cent sulfanilamide. Substantially the same results were obtained in several experiments. It is important to emphasize in this connection that complete inhibition of growth of certain strains of hemolytic streptococci in the presence of 0.8 per cent sulfanilamide cannot account for the effectiveness of this drug in the treatment of streptococcal infections, since such concentrations are not obtained in treated patients.

In order to determine whether sulfanilamide delays or completely inhibits the growth of hemolytic streptococci, various strains were incubated in 0.8 per cent sulfanilamide phenol red broth for a period of three weeks. These experiments revealed that with some strains no growth occurred within this period of incubation, although the controls showed profuse growth after eight to eighteen hours.

SUMMARY

1. The bacteriostatic action of sulfanilamide toward hemolytic streptococci is clearly demonstrable in a culture medium containing 1 per cent dextrose and maltose, respectively, 1 per cent soluble starch, phenol red broth base, and sulfanilamide.

2. Employing this culture medium it was found that:

(a) Sulfanilamide is bacteriostatic toward various strains of fibrinolytic hemolytic streptococci, but not, or only slightly, toward hemolytic enterococci isolated from man.

(b) Sulfanilamide in a concentration of 0.8 per cent may continuously inhibit the growth of susceptible strains, while smaller concentrations may retard their growth.

(c) The result as to the sulfanilamide susceptibility of hemolytic streptococci in the described order of experiment may be obtained after eight to twenty-four hours.

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A SIMPLE INEXPENSIVE TURBIDIMETER*

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SINCE a casual survey of the literature offered no description of a simple, inexpensive turbidimeter, it is thought that this note may be useful to other investigators. The apparatus described here is quite useful for rapid, approximate comparisons of turbidities, especially for bacteriologic work. Its value has been proved by use in standardization of bacterial spore suspensions, prepared under like conditions and employed in disinfection studies.[1]

Smith[2] describes a turbidimetric method somewhat similar in principle to the one described here. However, the Nessler tubes used in his apparatus are unsuitable for extensive bacteriologic work because of their expense, compared to that of test tubes, and their inability to withstand heat sterilization. Other turbidimetric methods described use either the more elaborate Tyndall effect or photoelectric cells. These methods require more expensive equipment, but are better adapted for more accurate work.

Although only corrugated cardboard has been used by the writer, the turbidimeter may be constructed of wood or metal if a more durable apparatus is desired. It hardly requires mentioning that the use of corrugated cardboard enables one to make this turbidimeter in a short time without the aid of a mechanic or tools other than a ruler and a sharp knife.

Fig. 1 shows the corrugated cardboard body, *A*, in light outline; a test tube rack, *B*, and light source, *C*, in heavy outline. Behind the test tube, *D*, shown in place, is a screen, *E*, which is made from a full-sized sheet (8.5 by 11 inches) of medium weight typing paper, on which is ruled in ink a series of parallel lines of various weights or widths. The lines are best made with a ruling pen and India ink. However, a fountain pen and ordinary black ink will serve the purpose. The screen is securely fixed to the back of the test tube rack with gummed paper. To simplify construction, the test tube rack, also consisting of corrugated cardboard, is made separately from the body, slipped through a slot in the top of the body, and then permanently fastened in place with gummed paper.

A longitudinal slot, cut in the top of the body, that just fits the light socket adds to the accuracy of the apparatus, as it allows partial regulation of the light intensity so that it is possible to determine which of two tubes is first to extinguish a selected ruled line. If no provision is made for varying the intensity of the light source, the apparatus is limited to a range of turbidities through which may be seen the ruled lines on the screen. But, if bulbs of different

*From the Bacteriological Laboratory, Mead Johnson and Co., Evansville.
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wattages or a rheostat in series with a bulb are used, the apparatus becomes adaptable to a range of turbidities, varying from almost clear to hardly translucent.

The dimensions given in Fig. 1 may be varied to accommodate the ruled screen and the test tubes used.

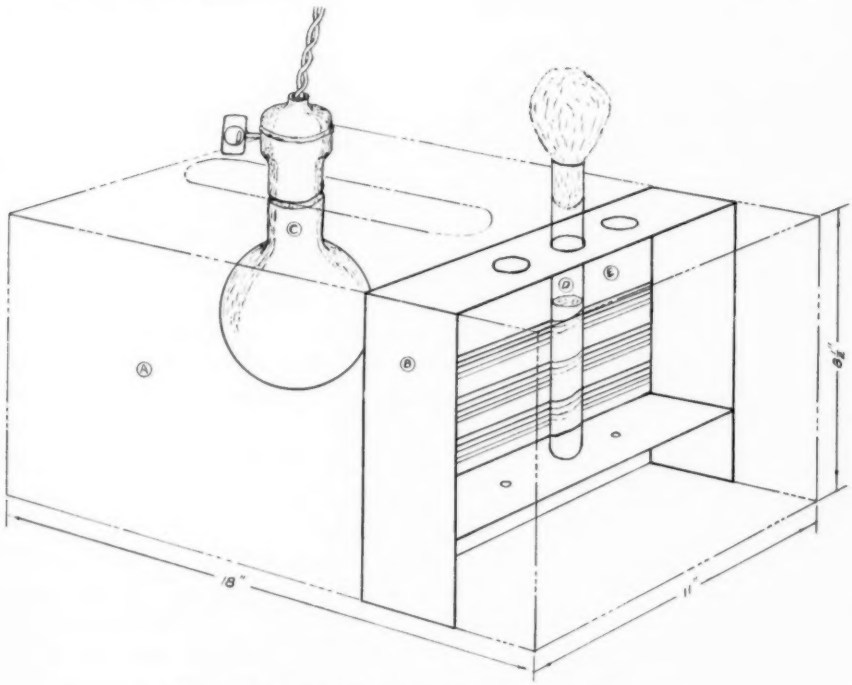

Fig. 1.—A phantom perspective of the turbidimeter.

Turbidity "standards" may be prepared from fuller's earth and distilled water, the proportions depending on the type of work to be done and the weight of lines used on the screen. The writer used one gram of fuller's earth to one liter of distilled water, allowed the heavier particles to settle for fifteen minutes, and prepared subdilutions from the resulting suspension by mixing 2, 4, 6, 8, and 10 c.c. of suspension with 8, 6, 4, 2, and 0 c.c. of distilled water, respectively. The prepared standards are sealed with rubber stoppers and can be kept indefinitely, if one is careful to shake them well before each determination.

A more simple utilization of this same principle may be realized by merely suspending the ruled screen between the light source and the test tubes. But, under these conditions, the glare around the edges of the screen is an interfering factor and only fair results may be obtained.

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SULFOCYANATE AS A TREATMENT FOR MANGE ON DOGS AND OTHER LABORATORY ANIMALS*

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FOR the past three years, we have been using a technical preparation of lauryl sulfocyanate, "Loro,"† as a treatment for mange on laboratory animals. The "Loro" contains about 50 per cent of active ingredients and an emulsifying and wetting agent which allows one to use it in aqueous solution. Since the substance was so effective in curing the mange on guinea pigs,[1] we decided to experiment with it on dogs and other laboratory animals.

A 5 per cent aqueous solution is applied by means of a clean rag or sponge while the dog is standing in a large pan. It is better to wash the dog before the treatment, but it is not absolutely necessary. It is not essential to apply the solution to the whole surface of the animal if there are only a few small spots that have been affected by the itch mites. However, if the affected spots are numerous and generally scattered over the body, it is well to wet the whole animal with the solution. Since the dog can and does lick a great part of its body, it is well to hold the dog for about thirty minutes after the treatment, or use a muzzle which will prevent him from licking off the active substance. If the mange is of long standing and there are open sores or ulcers, it may take two or more treatments, but usually two are sufficient. We have treated dogs that had lost practically all of their hair and apparently had their whole body surfaces affected by the itch mites. In all such cases, we have been able to clear up the condition. If two or more treatments are to be used, they should be given about a week to ten days apart.

This treatment has been given to dogs that have lived in two localities: Birmingham and University. The dogs from the latter city were pets and were treated by their owners who have reported in every case that the bare, thickened, and inflamed areas were cleared up, and that hair began to grow within a short time.

A few cats and rats have been treated for mange with the "Loro" and they too have been cured. It is much easier to use on rats than to follow the treatment for scabies which was suggested by Greenman and Duhring[2, 3]; "In case of badly infested ears it is more expeditious and quite as effective to trim off the affected edges."

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†E. I. du Pont de Nemours & Co., Wilmington, Del., supplied the "Loro."

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A NEW INSTRUMENT FOR TESTING CONDITION OF MYOCARDIUM AT AUTOPSY (MYOCARDIOMETER)

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IN A recent article,[1] I briefly described early experiments with a new instrument for testing condition of myocardium at autopsy, with especial reference to degree of myocardial degeneration. This report is presented because the instrument has been modified so that the readings are more accurately and more easily ascertained and the various degrees of degeneration are presented with cases listed under chief cause of death.

Death due to myocarditis or true inflammation is comparatively infrequent; whereas myocardosis or myocardial degeneration is found in most hearts of cases coming to autopsy. In deaths not due to cardiac causes, the heart may be only slightly degenerated. In deaths due to heart condition, they may be explained on a physical basis of intrinsic mechanical defect, congenital or acquired, such as marked valvular stenosis, regurgitation, congenital anomalies, etc., or because of the poor propulsive power of the heart due to marked softening and degeneration of the myocardium itself. Heart failure may occur with or without lesions of the valves. There is (1) right-sided, (2) left-sided, (3) total failure. The classical example of right-sided failure is mitral stenosis. Most of the cases reported here were either left ventricular failure or total failure.[2] Degenerations in the interventricular septum corresponded to degenerations of the left or right ventricular wall where there were no localized lesions due to coronary artery disease.

The myocardial fiber is a specialized cell, and is, therefore, subject to the usual degenerative changes from which cells suffer.[3] The arrangement of the musculature of the heart walls is such as to control with the greatest completeness the propulsion of the blood.[4] Degeneration of the myocardium occurs in many acute infectious diseases, such as diphtheria, scarlet fever, and others; it may be the result of poisons, such as phosphorus, arsenic, chloroform, ether, alcohol; and it may be due to anemia, primary or secondary. Grossly, the heart may be dilated and the muscle is likely to be soft, flabby, and pale. The cut surface bulges, and is more friable than normal. Practically never in infancy, rarely before the twentieth year, and increasing in frequency as age advances, the myocardium may show histologically numerous transverse fractures of the muscle fibers. It is usually stated that segmentation occurs in the line of the intercalated disks, and that a slightly different condition called fragmentation shows fractures of the fibers between and not necessarily involving the disks.[5] In segmentation the muscle fibers have separated at the cement line. In fragmentation the fracture has been across the fiber itself, and perhaps at the level of the nucleus.[6] Hearts the seat of marked fragmentation are lax, easily torn, the muscle fibers widely separated. In advanced cases of parenchymatous degeneration of the heart, it is very soft and friable. Softness means lessened

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resistance to palpation, friability means easy separation and division of muscle fibers by pressure with the ends of thumb and finger. These two conditions usually, though not necessarily, accompany each other but are different attributes. A heart muscle may be soft in early degeneration but not friable, that is, it will not yield and allow the fingers to make holes in it by simple straight pressure.[7] Therefore, the more degenerated the heart muscle, the softer and more friable it will be.[8] Using an instrument that measures the amount of force required to perforate a portion of myocardium, the more degenerated the myocardium, the less the force required, and hence the lower the instrument reading will be. The less degenerated the myocardium, the more the force required to perforate it, and the higher the reading will be. Marked localized

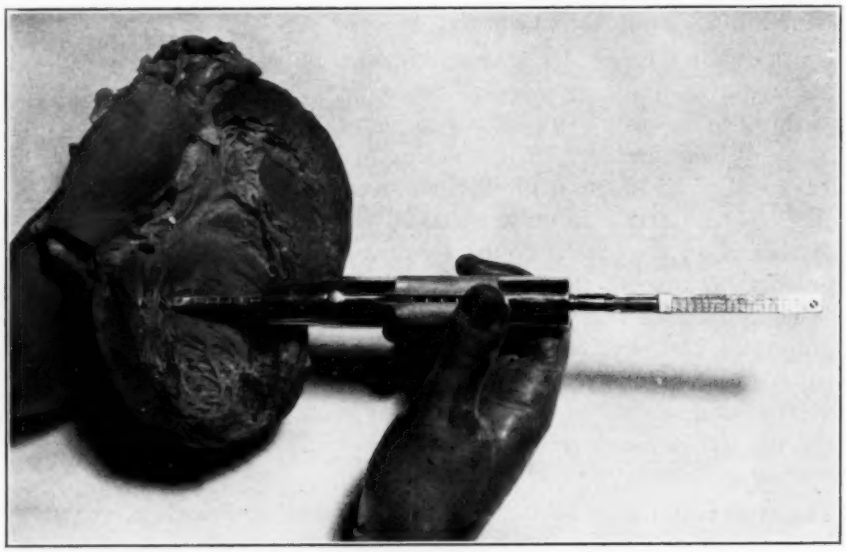

Fig. 1.—Performing test on heart muscle.

fibrosis would interfere with the accuracy of this test for myocardial degeneration. Large areas of localized fibrosis are usually due to coronary artery disease. Therefore, to perform the test in the heart, the center of the interventricular septum has been chosen because this area is least likely to show local changes due to coronary disease. This test is not designed to replace our previous methods of examining the heart at autopsy, but to add to our conception of the degree of myocardial degeneration. No longer is it sufficient to state that a heart shows myocardial degeneration; because a slight myocardial degeneration may but slightly have altered its function during life, whereas a marked myocardial degeneration must have altered the cardiac function to a greater extent. This instrument can be used at the time of autopsy in the gross examination of the heart to test degree of myocardial degeneration; so that one need not necessarily wait a week or more before histologic sections can be studied in order to determine the degree of degeneration. In most of the cases reported, the histologic examination of the cardiac muscle confirmed the gross findings of the myocardiometer.

Fatty degeneration arises from the same causes as, and is probably merely a later stage of, granular degeneration.[9] In cases of fatty degeneration of the heart, friability reaches an extreme degree, the wall being easily pinched through. In fatty infiltration of the heart, the affected muscle may be soft and friable but not to the degree of degeneration.

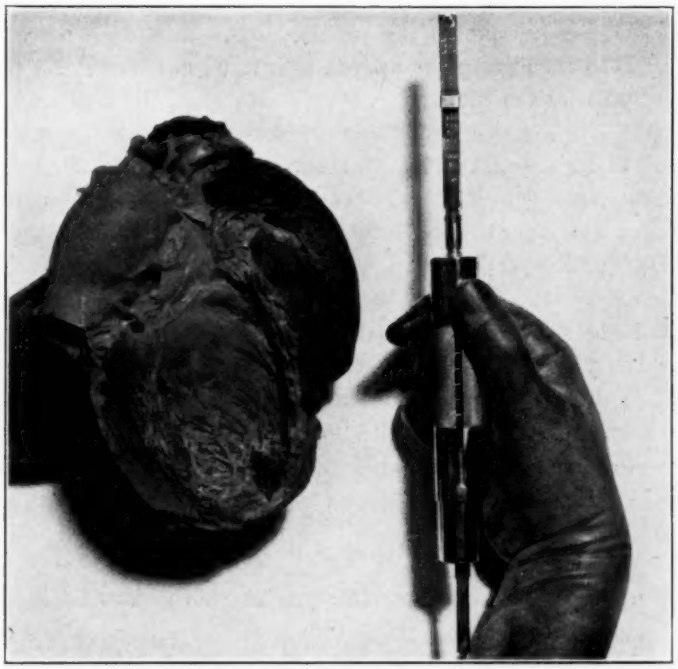

Fig. 2.—After test. Note reading in grams pressure.

The most marked degenerative changes in the heart muscle occur in necrosis.[10] Necrosis of muscle fibers in the myocardium may occur from cutting off the blood supply completely by embolism or thrombosis followed by infarction, or partially as the result of narrowing of the lumina of arteries by thrombosis, or in consequence of arteriosclerosis. It may also follow the effect of diffusible toxins (toxic myocarditis) or result from the immediate presence of infectious agents (infectious myocarditis). In necrosis of the myocardium, there is a lateral swelling and rupture of the fibers histologically.[10]

The lowest readings with the instrument (meaning the most marked degenerative changes) occurred in those cases that showed necrosis of the heart muscle histologically. Post-mortem changes play but a negligible part in the heart muscle from the standpoint of consistency. Examination of a heart removed from the body four hours after death, and examination again eight hours after death, showed exactly the same myocardiometer reading of 700 gm. Twelve hours after death, in a case of hypertensive heart disease with heart failure, the temperature of the myocardium was 86° F., and the myocardiometer reading was 800 gm. Thirteen hours after death the temperature of the myocardium was 79° F., and the myocardiometer reading was 800 gm. Fourteen hours after death the temperature of the myocardium was 75° F., and the myocardiometer

reading slightly above 800 gm. Even a variation of 50 gm. is of very slight significance, since there is a wide range of readings to indicate moderate degeneration, marked degeneration, etc. Autopsies done thirty-six to forty-eight hours after death gave the usual myocardiometer readings, depending on the degree of degeneration.

The instrument is made of brass, heavily chrome plated and rust proof. There are two brass cylinders, one telescoping within the other. Inside is a brass spring, 1/16 inch gauge, tempered to a fixed hardness. At the distal end is a plunger, 6.25 by 0.5 mm. wide, over 3 cm. long, divided into centimeters. The space from 1 to 2 cm. is subdivided into millimeters, so that the thickness of the interventricular septum can be measured between these two readings. Inside of the brass cylinders is a brass graduated rod that projects from the proximal end of the instrument. The end of this rod rests against an indicator pointing to 0. During the test, when the plunger is inserted into the heart muscle (interventricular septum), the spring within the tubes becomes compressed, and the graduated rod is pushed outward pushing the indicator ahead of it. After the plunger has pierced completely the cardiac muscle, the spring recoils, the rod drops back, but the indicator remains in position, having a tiny spring on one side to keep it in position. The indicator shows the number of grams required to perforate that cardiac area. The readings are from 0 to 2000 gm., being subdivided into hundreds of grams, e.g., 0, 200, 300, 400, 500, etc.

TECHNIQUE OF TEST

1. The endocardium is scraped away from both sides of interventricular septum of heart, exposing the myocardium. If the septum is thicker than normal, the muscle is cut away until it is 12-15 mm. thick. A difference of several millimeters thickness of muscle alters the reading but slightly.

2. Holding the heart in the left hand and the instrument in the right hand, the plunger is pushed slowly through the center of interventricular septum.

3. When the plunger perforates the heart, the spring recoils, leaving indicator in position.

Reading is made in grams pressure required to perforate myocardium.

The more degenerated the heart muscle, the softer and more friable it is, and the lower the reading will be.

It is suggested that the degrees of degeneration be graded from grades 1 to 4.

	Gm.	
Normal hearts (accident cases, etc.)	1800	
Slight degeneration	1300-1800	Grade 1
Moderate degeneration	900-1300	Grade 2
Marked degeneration	600- 900	Grade 3
Very marked degeneration	Below 600	Grade 4

Readings of 1300 are considered as moderate degeneration.
Readings of 900 are considered as marked degeneration.

When the reading is 900 or below, congestive heart failure has usually taken place, and in the absence of marked changes in the other vital organs, it is considered to be a cardiac death due to advanced myocardial degeneration.

A total of 145 cases is presented, 78 males and 67 females.

Grade 3 and grade 4 myocardial degeneration is severe enough to have caused death, in the light of our present knowledge. Grade 2 myocardial degeneration may have been a cardiac death, if there were marked local degenerative changes due to coronary artery disease, or if there were intrinsic mechanical defects, such as marked stenosis, congenital anomalies, etc.

Of the 145 cases, the hearts showed the following degrees of degeneration: 5 normal, 31 slight degeneration, 58 moderate degeneration, 38 marked degeneration, and 13 very marked degeneration.

Myocardiometer Readings (grams pressure)

Normal Hearts

Skull fracture	1800 1800 1800	Spleen rupture 1900
Brain tumor	1800	

Hypertensive Heart Disease

Benign hypertension,				Malignant hypertension,				Chronic glomerular nephritis		
1500	1300	1000	1350	800	1450	700	1100	800	700	1000

Acute Rheumatic Heart Disease

900 1150 1300

Coronary Thrombosis

700 900 1100 900 1100 1250 1200 800 800

Subacute Bacterial Endocarditis

1300 700 1350

Myocardosis (due to toxic factors in chest, abdomen, brain, etc.)

1600	800	800	600	1300	1500	1700	1000	1500	1600	1450	1500
1000	500	600	800	1300	900	600	600	900	1300	1450	900
1100	750	800	900	1600	1300	1150	700	900	1100	1300	1250
1000	600	800	600	1300	800	Gas bacillus septicemia				400	450

Carcinoma

Breast	1600	1300	900			Head of pancreas			1100	1250	1100
Uterus	1500	1100	1200			Lung			1500	900	
Prostate	1300	1600	1100	600	800	Liver (primary)			900		
Urinary bladder	1300			Stomach		700	900	1500	1450	1100	1100	1300
Testicle	1300			Large intestines					1500	1500	1050	1450

Sarcoma

Bone 1300 1500 Lymphosarcoma 1200

Lobar Pneumonia

1100	1300	1100	1100	1500	1800	1100	1300	1150	1500	1500	1000

Bronchopneumonia

700	600	700	900	900	1100	1100	1100	1200	1300	1200	900	900	900	900	1100	1150	1100	1200

Pulmonary Tuberculosis

1800 1400 1700 1500

Acute Myelogenous Leucemia

550

Agranulocytic Angina

600

To summarize it, hypertensive heart disease showed grade 1, 3 cases; grade 2, 4 cases; grade 3, 4 cases. Acute rheumatic heart disease showed grade 2, 2 cases; grade 3, 1 case. Coronary thrombosis showed grade 2, 4 cases; grade 3, 5 cases. Subacute bacterial endocarditis showed grade 1, 1 case; grade 2, 1 case; grade 3, 1 case. Carcinoma showed grade 1, 10 cases; grade 2, 16 cases; grade 3, 6 cases; grade 4, 1 case, this last case showing extensive suppuration within the stomach malignancy. Lobar pneumonia showed grade 1, 4 cases; grade 2, 8

cases. It is interesting to note here that not a single case of the 12 lobar pneumonias showed marked or very marked myocardial degeneration. Bronchopneumonia presented a different picture, grade 2, 10 cases; grade 3, 8 cases; grade 4, 1 case. Pulmonary tuberculosis showed grade 1, 4 cases.

SUMMARY

A new instrument has been devised to test condition of heart grossly at autopsy, thus aiding in a more accurate way our conception of the degree of degeneration of the heart muscle.

Histologic examination of sections of heart from the site of test corroborated the gross findings as to degree of myocardial degeneration.

One hundred and forty-five cases were studied at autopsy, 78 males and 67 females.

A much greater series of cases must be studied in order to ascertain the degree of degeneration in various types of diseases.

It is suggested that slight myocardial degeneration be called grade 1 degeneration; moderate, grade 2; marked, grade 3; very marked degeneration, grade 4. Grades 3 and 4 myocardial degeneration can be assumed to be cases of primary cardiac death in the absence of marked pathology in the other vital organs (lung, brain, etc.).

Average heart readings showed less myocardial degeneration in lobar pneumonia than in bronchopneumonia. This applied to the cases of bronchopneumonia which preceded cardiac failure and were not terminal bronchopneumonias on a pre-existing pulmonary congestion and edema.

Average heart readings showed slight or very slight degeneration in pulmonary tuberculosis.

The lowest myocardiometer readings, with the most marked degree of generalized myocardial degeneration with necrosis of heart muscle, were found in two cases of gas bacillus septicemia (*B. aerogenes capsulatus* or *B. welchii* infection).

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The cases included in this report were from the Jewish Hospital, Philadelphia, and Temple University Pathology Department.

I wish to thank Dr. L. W. Smith, Dr. J. C. Doane, Dr. E. S. Gault, Dr. F. Konzelmann, Dr. H. C. Lennon, and Dr. E. E. Aegerter, for their helpful suggestions and some of the figures.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

MONONUCLEOSIS, Infectious, False Positive Wassermann Reaction in, Bernstein, A. Am. J. M. Sc. **196:** 79, 1938.

Transitorily positive serologic tests for syphilis were encountered in 6 out of 37 cases of infectious mononucleosis. During the acute stage of the disease the Wassermann test, the Eagle test, or both reactions, became positive to high titers, but spontaneously reverted rapidly to negative. These positive tests are independent of the presence of sheep-cell antibodies in the patient's serum and indicate, as does the occasional occurrence of miscellaneous bacterial antibodies, the versatility of antibody responses in infectious mononucleosis. In any instance of apparent purpura hemorrhagica with a false positive Wassermann reaction, a Paul-Bunnell test should be performed in order to establish the possible diagnosis of the thrombocytopenic variety of infectious mononucleosis. Reasons are stated for suspecting that the causative agent of infectious mononucleosis may be a protozoon.

BLOOD, New and Simple Method for Detection of, by Heating, Moody, A. M., Proescher, F., and Carr, J. L. Arch. Path. **26:** 501, 1938.

Study of the physical changes which occur when blood and a few other organic substances are heated to high temperatures has demonstrated that dried blood melts at from 500° to 515° F. (260° to 268° C.), and that blood after further heating above 700° F. assumes a specific structure. This structure is easily identified in quantities as small as 1 mg., even when the blood has been mixed with inorganic or other organic substances. The physical changes are specific, not only for blood, but also for the degree of heat used.

SPUTUM, Quantitative Method for Estimating the Number of Tubercle Bacilli in, Jordan, E. F. Am. Rev. Tuberc. **38:** 241, 1938.

The sediment from the centrifuge tube in which the mixture of sputum and 4 per cent sodium hydroxide had been spun for thirty minutes at high speed is washed with sodium chloride solution into a small test tube, which has a volume of 1.5 c.c. indicated by a file mark. The centrifuge tube is twice rinsed with a small amount of salt solution, and the rinsings are added to the sample. The volume of the combined sediment and rinsings is then made up to 1.5 c.c. by the addition of saline solution. Three or four glass beads are added to the tube which is then stoppered and agitated in the shaking machine for thirty minutes. (Others in the laboratory have found that a hard shaking by hand for five minutes is equally satisfactory.) The slides are marked, as Hughes suggests, with a diamond pencil line drawn 15 mm. from one end across a slide 1 inch wide. After cleaning with acid alcohol, a loopful of sterile serum is spread over the area marked off. The serum dries almost immediately and serves as a fixative. A measured amount of the digested concentrate is added, using a pipette graduated in hundredths of a cubic centimeter. This is spread as evenly as possible over the layer of serum. For the first count, four slides are made from each specimen with 0.02, 0.04, 0.06, and 0.1 c.c., respectively. This is done in order to obtain a slide in which there are 10 to 25 bacilli in each field. Fixing and staining are carried out in the usual manner.

In examining the slides under the microscope, a preliminary inspection is made to ensure that there is a fairly even distribution and that the slide is free from clumps of bacilli which in some specimens are especially troublesome. Elastic tissue may enmesh an enormous number

of microorganisms and prevent an accurate count, and occasionally bacilli are grouped as if agglutinated. Of the 38 concentrates examined, 3 were found to be uncountable for one of the above reasons.

If the slides were not obviously uneven, two groups each of ten fields were counted. The computation of these results is very simple. The mean of ten fields is determined. If the slide was made with 0.02 c.c., the mean is multiplied by 75; if 0.04 c.c. was used, 37.5 is the factor; if 0.06 c.c. was used, 25 is the factor; and for 0.1 c.c., 15, which gives the number of microorganisms per microscopical field in 1.5 c.c., is the volume of the concentrate. The number of microscopical fields in the area on the slides is determined, as in the Hughes method, by establishing the diameter of the field in use with a hemocytometer and solving the formula $\frac{1}{4} \pi D_2$ for the area of the microscopical field. The area of the slide which the diluted concentrate is spread over is 25.4 mm. by 15 mm., or 381 sq. mm. $\frac{1}{4} \frac{381}{\pi D_2} = 31046$, the number of microscopical fields per slide. This must of course be determined for each microscope. For simplicity we have used 31,000, so our formula is:

$$\text{Mean} \times \frac{1.5}{\text{Amount of material used on slide in c.c.}} \times 31{,}000 = \text{Number of bacilli in sputum.}$$

Since the concentrate contained the tubercle bacilli from a seventy-two-hour sample, the result of the formula is in terms of the excretion for that period. It was not converted into twenty-four-hour excretion because we wished to use the Gaffky on these specimens for comparison.

ENDOCARDITIS, Experimental Streptococcic, Kinsella, R. A., and Muether, R. O. Arch. Path. **62**: 247, 1938.

Seventeen dogs were subjected to operation whereby the mitral valve or the chordae tendinae were cut. All these animals were then fed with living cultures of nonhemolytic streptococci either mixed with food or by stomach tube. Ten of the animals became sick, displayed positive results of blood culture, and died. At autopsy these infected animals had bacterial endocarditis. The bacteria in the vegetations were determined to be identical with those that had been fed to the animals.

Streptococcic endocarditis can be produced in dogs with injured cardiac valves by feeding them streptococci.

The fact is thus established that bacteria entering the animal body through the mouth may become implanted on an injured area within the body. The exact route which these bacteria follow is not determined.

The reproduction of streptococcic endocarditis is complete. The success of two different drugs in curing the disease in dogs, while failing to cure the disease in human beings, does not obscure the identity of the experimental disease. On the other hand, the difference in the mode of production of the experimental disease and in the mode of production of the disease in human beings is emphasized. This difference lies in the fact that bacterial implantation begins after an injury produced by trauma in the one instance and as a result of disease in the other. It seems highly important to collect a series of animals cured of streptococcic endocarditis and then to study the treatment after reinfection of the healed scars of previous infection. This will be a tedious task. The most interesting by-product of the present study has been the demonstration of infection of traumatized valves by means of feeding streptococci to the animal. This part of the work, repeated in different years, seems adequately authenticated. It is of further interest that none of the microscopic appearances in the heart or elsewhere was such as to suggest a relation between the lesions observed in dogs and those of rheumatic fever which appear in human tissues.

ANEMIA of Alcohol Addicts, Bianco, A., and Jolliffe, N. Am. J. M. Sc. **196**: 414, 1938.

A quantitative study of the red blood cells in 184 cases of alcohol addiction is reported. Of these subjects, 159 were "complicated" in that they had, in addition to alcohol addiction, one or more of the following diseases: polyneuritis, pellagra, "alcoholic" stoma-

titis, ''alcoholic'' encephalopathy, or liver cirrhosis; 25 of these subjects were ''uncomplicated'' in that they had none of the above listed diseases or other recognized stigmas of chronic alcoholism.

In these subjects, quantitative anemia did not occur in the ''uncomplicated'' group, but was present in 61 per cent of the ''complicated'' group. However, macrocytosis was present in about 50 per cent of both groups. Macrocytic anemia in these alcohol addicts was not limited to subjects manifesting pellagra or cirrhosis of the liver, but occurred as well, and in about the same frequency, in subjects having polyneuritis, ''alcoholic'' stomatitis, and ''alcoholic'' encephalopathy. There was also no correlation between the frequency of macrocytosis and achlorhydria, severity of liver damage, or the presence of an enlarged liver. In view of these findings the authors are inclined to regard the macrocytosis of the alcohol addict not as a manifestation of inability on the part of the liver to store a hematopoietic principle, but as an extrinsic deficiency of some necessary hematopoietic substance required to maintain normocytosis.

E. COLI, Ingestion of Red Blood Cells by, and Its Significance in, Tyzzer, E. E., and Geiman, Q. M. Am. J. Hyg. **28**: 271, 1938.

The present study was initiated by a problem in diagnosis arising from a case of brief intestinal obstruction which presented a discharge of blood-streaked mucus in which were numerous endamoebae with ingested red blood cells.

A thorough study of the amoebae present, taking into account morphology of trophozoite and cyst, growth on culture media, and the results of animal inoculation, failed to furnish any evidence of the presence of *E. histolytica*, but showed conclusively that red blood cells were being ingested by *E. coli*. On consideration of pathologic conditions other than amoebiasis to account for the continued presence of blood in the stool, a papillomatous polyp of the sigmoid was located and removed.

The observations of Dobell in regard to the ability of *E. coli* to ingest red blood corpuscles in vitro, find application in the occurrence, under certain circumstances, of red blood cells in this species as it is passed from the human intestine.

The acceptance of the presence of amoebae with ingested red blood cells as sufficient, without further morphologic study, for a diagnosis of amoebic dysentery may be expected to lead to erroneous diagnosis involving not only failure to recognize conditions actually present but also the subjection of patients to costly and unnecessary treatment.

WHOOPING COUGH, Diagnosis of, Donald, A. B. Brit. M. J., Sept. 17, p. 613, 1938.

An attempt has been made to assess the value of the various methods available for the early diagnosis of whooping cough. The methods are: 1. Specific tests—(a) cough-plate method, (b) complement fixation tests, (c) intradermal tests; 2. Non-specific tests—(d) total and differential leucocyte count; 3. Erythrocyte sedimentation rate.

Specific Tests.—(a) The cough-plate method is the only means of certain diagnosis in the earliest stages of the disease. In a series of 136 cases at all stages, 100 per cent were found to give positive plates in the first week, 93.5 per cent in the second week, 94.8 per cent in the third week, and 44 per cent in the fourth week, after which there is a rapid fall to 7.1 per cent in the fifth week, and less than 1 per cent in subsequent weeks. The value of the cough plate in the diagnosis of atypical and abortive cases is stressed, and its value to public health administrators is mentioned. Emphasis is also laid on the negative cough plate as a criterion of the termination of infectivity.

(b) The complement fixation test is shown to be of little value except as a means of corroboration of the diagnosis, and occasionally as an indication of immunity. In a series of 123 cases the reaction was found to become positive in 25 per cent during the third week of illness, and reached a maximum during the eighth week, when 89 per cent of the cases gave positive fixation reactions.

(c) The intradermal test, using a suspension of *H. pertussis*, is found to be valueless in diagnosis or as a method of determining susceptibility or immunity.

Non-Specific Tests.—(d) The total and differential leucocyte count is found to be valuable from the second week onward, almost all cases examined showing marked leucocytosis and both absolute and relative lymphocytosis. During the third and fourth weeks the leucocyte count averaged 30,000 per c.mm., with a differential count showing 65 per cent lymphocytes and 28 per cent polymorphonuclears.

(e) The erythrocyte sedimentation rate is shown to be slightly retarded or normal in almost all uncomplicated cases of whooping cough in the early stages, with a slight increase in the later stages of the illness.

ALCOHOL in Relation to Traffic Accidents, Holcomb, R. L. J. A. M. A. **111**: 1076, 1938.

The highest percentage of drinking drivers occurs in the early morning and over the week-end.

The largest number of drinking drivers occurs in the early evening and over the week-end.

The peak age for drinking drivers is from 25 to 30.

Women drink and drive as much as men when the number of women driving at various hours of the day is considered.

The percentage of drinking drivers in the general population varies as does the percentage of drinking drivers in the personal injury accident group, but falls considerably lower at all times.

The percentage or number of drivers involved in personal injury accidents varies as does the percentage or number of drinking drivers.

As the blood alcohol content increases, the number of drivers appearing in the personal injury accident group increases out of all proportion over that in the general driving population.

As alcohol increases, accidents increase and at a rate somewhat proportionate to the increase in alcohol.

Equal percentages of drinking drivers are found in the accident group and in the general population group at a point near 0.5 part of alcohol per thousand parts of blood, indicating that alcohol in that amount is not necessarily a significant cause of accidents.

It has not yet been objectively and conclusively proved just how important a causative factor alcohol is, and, because of the complexity of the whole accident problem, it may never be proved. The data gathered in this study, however, point in one direction only. They confirm a self-evident fact, that alcohol is a major cause of automobile accidents.

TISSUE: Combined Gram-Methyl Green-Pyronine Stain for Formaldehyde-Fixed Tissue, Lisa, J. R. Arch. Path. **26**: 728, 1938.

Technique.—Paraffin sections of tissue fixed in 10 per cent solution of formaldehyde U.S.P. are prepared as usual for staining.

1. Stain with crystal violet solution for five minutes. Wash thoroughly.
2. Place in alkaline iodine solution for five minutes. Wash.
3. Decolorize with pure technical acetone. Wash.
4. Stain with methyl-green-pyronine solution for from one to two minutes. Wash.
5. Decolorize with acetone, clear in two changes of xylene, and mount in balsam.

Preparation of Solutions.—Crystal violet, 1 gm., is dissolved in 10 c.c. of buffer phosphate solution of pH 6.6 to 7 and diluted to 100 c.c. with distilled water.

Resublimed iodine, 2 gm., is added to 10 c.c. of normal sodium hydroxide diluted to 30 c.c. The iodine solution is shaken frequently and gradually diluted to 100 c.c.

In a flask is placed methyl green, 0.65 gm., and a special pyronine, 0.1 gm. Hot distilled water, 100 c.c., is added to the dry dyes, and the solution allowed to stand for from three to four days before being used. It is stored in a well-stoppered amber glass bottle. The methyl green used is more highly ethylated than the usual grade and has a dye content of 65 per cent. The pyronine is a special yellow pyronine.

All the solutions can be made up in bulk, since they are stable. In this laboratory, at present, they are made up in 3 liter volumes. When the larger amounts are prepared, only the first half of the distilled water used in dissolving the methyl green and pyronine needs to be heated.

The length of staining is important. Not less than five minutes is necessary for both the crystal violet and the iodine. A shorter time tends to cause uneven staining of the gram-positive organisms, resulting in inaccurate observations. A short period only is advisable for the counterstaining, from one to two minutes. It is occasionally better to dilute the counterstain, particularly when the cellular exudate is dense. The dilution can be modified to suit the particular requirement for depth of color.

The acetone must be of an excellent grade; any impurity vitiates the results, particularly in regard to the counterstain. After the first decolorization, the acetone should be washed off quickly, without allowing the tissue to dry. Decolorization of the counterstain must be carried out promptly after washing. If the slide is allowed to stand in water, the counterstain tends to become homogeneous in color, and the differential characteristics are lost. This does not apply to the washing after staining with iodine, since allowing the slide to stand in water several minutes causes no difficulty. After the second decolorization, plunging the slide into xylene as soon as the tissue dries facilitates clearing.

Gram-positive bacteria stain deep purple black. Gram-negative bacteria stain magenta. The Neisserian organisms particularly stain brilliant deep magenta. Tubercle bacilli do not stain, but lepra bacilli stain like other gram-positive bacteria. Capsules either are unstained or faint lavender. Polymorphonuclear cells have nuclei varying from dark purple to green blue; their cytoplasms show a faint, finely granular lavender. Small lymphocytes have dark navy blue nuclei and an unstained or faintly magenta cytoplasm. The nucleus of the plasma cell is dark navy blue, with a cartwheel arrangement of the chromatin; the cytoplasm shows a homogeneous deep magenta except in a clear perinuclear zone. The eosinophiles have nuclei similar to those of the polymorphonuclear cells, the granules stain somewhat unevenly reddish or purple. The granules of the mast cells stain like gram-positive cocci but are easily distinguished by their homogeneous shape and their arrangement, obscuring the nucleus. Endothelial cells have very pale blue, delicate nuclei. Giant cells of the Langhans type have nuclei similar to that of the endothelial cell; their cytoplasm stains like that of the plasma cell. The Aschoff cell is similar. Erythrocytes are bronze to yellow.

LYMPHADENOPATHY, Giant Follicular, With or Without Splenomegaly, Symmers, D. Arch. Path. **26**: 603, 1938.

Giant follicular lymphadenopathy with or without splenomegaly is a clinical and pathologic entity. Clinically, it resembles Hodgkin's disease, certain forms of lymphosarcoma, lymphatic leucemia and the like. Histologically, the changes are characteristic and consist in numerical and dimensional hyperplasia of the lymph follicles of lymph nodes and spleen. The condition is probably of "toxic" or "inflammatory" origin and is usually amenable to mild roentgen therapy.

Giant follicular lymphadenopathy is capable of direct transformation into a heretofore undescribed polymorphous cell sarcoma having multiple foci of origin in hyperplastic lymph follicles.

Giant follicular lymphadenopathy with splenomegaly is capable of assuming the histologic features of Hodgkin's disease as added associated phenomena and not as a process of transformation. It may likewise become associated with the changes of lymphatic leucemia.

The term "reticulum cell lymphosarcoma" should be abandoned as misleading. No such tumor has been shown to exist. The tumor so designated is in reality the large round-cell sarcoma of lymph nodes and is to be distinguished from the polymorphous cell sarcoma of lymph follicles discussed in this paper.

The disease described under the histologic designation "necrotic folliculitis of lymph nodes and spleen" is believed to be unique in that, among other things, it represents a heretofore unrecorded phase in the evolution of giant follicular lymphadenopathy with splenomegaly.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Medical Applications of the Short Wave Current*

IN THIS book Bierman and Schwarzschild present a clear, sane, and logical exposition of the fundamental aspects of short wave therapy and an excellent and comprehensive discussion of its clinical applications.

Manual of Veterinary Bacteriology†

THE third edition of this excellent manual deserves as cordial a reception as its predecessors and takes their place as the standard reference text in this field. Lieut. Col. Kelser's book can be heartily recommended.

The Synovial Membrane and the Synovial Fluid‡

IN THIS book, the author, formerly research fellow in the Hospital for Joint Diseases, New York, presents the digested results of fifteen years' research on the structure of the synovial membrane, the origin and nature of the synovial fluid, and the composition of pathologic joint effusions.

Part I (Chapters I-VII) is devoted to a discussion of the synovial membrane. Part II (Chapters VIII-XIV) discusses the synovial fluid; and Part III (Chapters XV-XXIV) discusses clinical applications.

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*Medical Applications of the Short Wave Current. By William Bierman, M.D., Attending Physical Therapist, Mt. Sinai Hospital, New York City. Including a Discussion of the Physical and Technical Aspects. By Myron M. Schwarzschild, M.A., Physicist, Beth Israel Hospital, New York City. Cloth, 379 pages, 25 plates, $5.00. William Wood & Co., Baltimore, Md.

†Manual of Veterinary Bacteriology. By Raymond A. Kelser, D.V.M., A.M., Ph.D., Lieut. Col. Vet. Corps U. S. Army; Chief, Veterinary Division, Surgeon General's Office, War Department, Washington, D. C. Cloth, 640 pages, 93 illustrations, $6.00. Williams & Wilkins Co., Baltimore, Md.

‡The Synovial Membrane and the Synovial Fluid, With Special Reference to Arthritis and Injuries of the Joints. By David H. Kling, M.D., Assistant Professor of Orthopedic Surgery and Chief of Arthritis Clinic, White Memorial Hospital, Los Angeles, Calif. Cloth, 299 pages, 80 illustrations, $5.00. The Medical Press, Los Angeles, Calif.